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OCTOBER, 1931.

A SPECIAL PHYSIOLOGY NUMBER.

In our last issue we drew attention to the absence of opportunity for veterinary research in the subject of animal physiology, and the want of collaboration between those who work upon animals in the physiological laboratories attached to our human medical and other scientific schools, and the veterinary practitioner, whose opportunities for putting into beneficial practice the results which the laboratory scientist attains could be of such value to the sick animals themselves, and a godsend to himself.

It is a standing disgrace to Great Britain that none of its veterinary colleges possesses a physiology chair which is filled by a graduate in veterinary science. Such an anomaly could not exist in any other country but ours, and it is not at all a matter of surprise when one finds that almost all the beneficial discoveries for the treatment of disease, which have involved physiological research, have their origin abroad. All veterinary colleges on the Continent and in America have well-equipped physiological laboratories, and the work done in them is undertaken by men who have a veterinary foundation; their scheme of work being mapped out with the idea of its application to the alleviation of suffering and the prevention or cure of disease in animal patients.

There is very much to do, in fact an enormous field is open, in this direction, and if we veterinarians do not assert our rights we shall not only lose our opportunity, but our animal patients (healthy as well as sick, large as well as small) will continue to suffer from the lack of knowledge which undoubtedly exists because the subject of animal physiology is not only insufficiently taught, but inadequately applied.

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General Articles

A BRIEF NOTE ON THE DIAGNOSIS OF EQUINE PREGNANCY BY BIOLOGICAL TEST.

By F. A. E. CREW, Wm. C. MILLER,
AND
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Introduction.

The pioneer work of Aschheim and Zondek upon the hormone content of the urine of pregnant women (1927) has aroused the greatest activity, and it is now established that such urine, in virtue of its hormonal constituents, provokes two characteristic changes in the ovary and vaginal epithelium of the female mouse. When injected into a sexually immature female mouse it leads to the production of characteristic blood spots—*corpora atretica*—in the ovary, and when injected into an ovariectomised adult female mouse it induces a well-defined and characteristic change in the structural features of the epithelium of the vagina. Full advantage has been taken of these facts in order to devise a test for pregnancy in the human female. The Zondek-Aschheim test for human pregnancy can yield positive information as early as the tenth day after conception. In this country a human pregnancy diagnosis station was established at this Institute some three years ago. It was devised for the provision of reliable service for the medical profession, and it is now used to such a degree that it is a self-supporting mechanism. From all over the country samples of urine to be tested are sent in, and in the course of the year about 800 reports are despatched within about ninety-six hours after receipt of the sample.

In 1927, Zondek and Aschheim demonstrated that similar effects could be produced by the use of the urine of pregnant cows, and since that time the work has been extended to include other animals of economic importance. During the past twelve months work has been in progress in this Institute to determine whether or not the pregnancy diagnosis test could be used in the case of the mare. A small herd of Shetland ponies formed a basis for preliminary investiga-

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tion, while more extended results were made possible by personal contact with a large number of practising veterinarians, and by the circulation of a request that others should forward samples for diagnosis. The response to these requests has been good; samples have arrived from many parts of this country, from France, and India.

Collection of the Sample.

The technique employed for collecting the urine is exceedingly simple. Normally, with the mares kept at the Institute, each sample was secured by the use of a catheter, employing a vaginal speculum in the smaller Shetland ponies. Otherwise, it is a simple matter to instruct a groom or horseman to collect some 50 c.c. in any clean wide-mouthed bottle, by watching for an opportunity when a mare passes urine on coming in from work or exercise.

The writers encountered a certain amount of prejudice against the use of a catheter in the pregnant mare by practitioners. Undoubtedly there may be some small risk of inducing abortion by careless catheterisation of a heavily-pregnant mare, especially if the catheter is forced against, or even into, the os of the cervix. Such cases, however, apart from other considerations, require no diagnosis by biological test. We think it would be almost impossible to cause abortion by simple catheterising during the earlier stages of pregnancy. It may be mentioned in passing that the ponies which were used in the earlier stages of this investigation were unbroken, most had only been haltered with difficulty, and several were only two years old, when they arrived. Apart from the occasional use of a twitch for a fractious animal, no restraint other than a halter was used, and no technical difficulty was ever encountered. Even after the passage of a catheter two or three times weekly for months at all stages of pregnancy, no complications whatever ensued. It should be pointed out, however, that occasionally when the bladder has been emptied just previously, it may be impossible to induce more than a few drops of urine to escape from the end of the catheter.

We have followed the rule of giving instructions for samples to be despatched as soon as practicable, though it appears probable that the hormone content of the urine does not rapidly depreciate, in view of the results obtained from samples from India, which were three to four weeks in transit. We prefer, however, to reserve a definite opinion on this point in the meantime. In a few instances we have made use of a rubber urine-collecting apparatus, specially devised for the purpose, where it was desirable to obtain a large amount of urine during the twenty-four hours.

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Brief Description of the Test.

It was our experience here, and it has been found by many research workers, that the injection of untreated equine urine into mice was frequently followed by death. This urine has normally a high mucin content, and its protein content is considerable. In order not to vitiate the test, it is necessary to precipitate the protein bodies by adding a solution of sulphosalicylic acid; this also possesses some antiseptic action, and, it has the advantage that it does not prejudice the activity of hormones present. After filtration the sample is neutralised with sodium bicarbonate, using B.D.H. Universal Indicator.

It was early recognised that the diagnosis of pregnancy in the mare would best be determined by the effect upon the vaginal epithelium of the mouse. The effect upon the ovary, which would seem to be due to a different hormone, is in the case of the mare not nearly so intense and characteristic. At the present time, therefore, the test which is used is that which refers only to the induced cornification of the vaginal epithelium.

To obviate the possibility of any stimulation which the injected urine might have upon the mouse's own ovaries, and which might result in hormone production by them, ovariectomy is performed upon each of the test animals, usually at the age of four to five weeks, and at least eight days previous to the commencement of the test. After a further period of rest lasting for a minimum of one week, to allow the vaginal epithelium, which may have undergone partial or complete cornification, time to return to normal, the mice may be used again and again until four to five months old.

For ordinary test, four mice are used. The total amounts of urine injected range from 1.2 c.c. in the first, to 3.0 c.c. in the fourth mouse; one sixth of these amounts is given to each mouse night and morning for three successive days. On the fifth day after the commencement of the test, vaginal smears are made from each mouse, stained (Giemsa) and examined microscopically.

Interpretation of Results.

The injection of œstrus-inducing hormone into ovariectomised mice induces those vaginal changes which are associated with œstrus in the mouse. During the œstral cycle in the mouse there is a fluctuation in size of the uterus and vagina, and a change in the vaginal epithelium. During the rest or diœstrus stage the uterus is small and contracted, during pro-œstrus an increase in size, due to the accumulation of fluid, takes place, reaching its maximum during œstrus, while subsequently, in metœstrus, regression to normal occurs.

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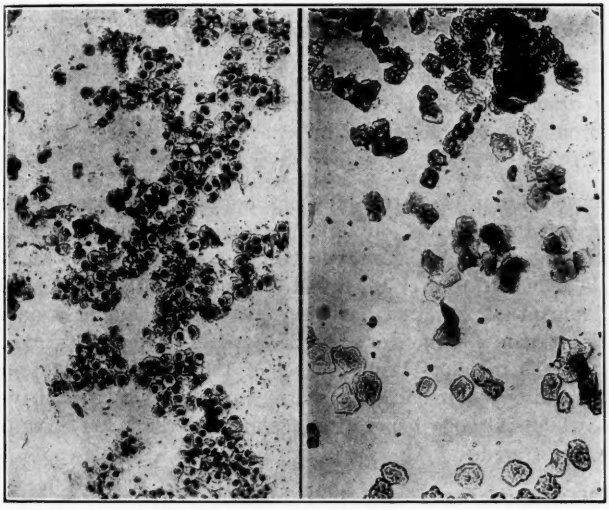
The cells of the vaginal epithelium during diœstrus are nucleated and cuboid, and stain well, while mixed with them are a certain number of leucocytes. At the beginning of pro-œstrus, only typical nucleated epithelial cells are found, and these change to cornified cells as œstrus approaches. At the height of œstrus only cornified cells can normally be demonstrated in a vaginal smear (*see* illustration). Leucocytic invasion occurs as œstrus passes into metœstrus, at which stage a large number of leucocytes occupy the smear. Subsequently, cubical nucleated cells appear as the diœstrus stage is once more reached, the leucocytes gradually diminishing in numbers.



A B

TYPICAL VAGINAL SMEARS.
A—showing nucleated cubical epithelial cells—diœstrus.
B—showing cornified cells—œstrus.

For the purposes of the test under discussion, only a fully cornified vaginal epithelium is regarded as evidence of a positive reaction. Incomplete cornification may readily result from the injection of urine from a mare which is non-pregnant, but which was in one or other phase of the œstrous cycle herself when the urine was taken.

Fluctuations of Hormone Concentration.

Undiluted urine which had been detoxicated and neutralised, and which, when injected in the appropriate amounts into each of four

mice, gave cornification of the vaginal smear in each, was considered at first to have come from a pregnant mare, and the test was regarded as positive. The strength of the hormone which is required to give this reaction is 800 mouse units per litre of urine. We now know, however, that in certain cases enough œstrus-inducing hormone may be excreted in the equine urine during œstrus, or about the time of œstrus, to give this apparently positive reaction.

It is now our custom to dilute the urine by admixture of varying amounts of normal saline in order to reduce the concentration of the œstrus-inducing hormone, so that it will be possible to estimate the amount of M.U. per litre in a given sample. It is possible to suggest that no concentration of less than 1,000 M.U. should be regarded as positive evidence of pregnancy, and that with concentrations of less than 1,000 M.U., but more than 800 M.U., a second sample should be tested after an interval of two to three weeks. The largest concentration from a known non-pregnant animal so far obtained has been 833 M.U.

Concentration of œstrus-inducing hormone rises as pregnancy proceeds. In one case, on the ninety-fifth day after service, the excretion was 2,500 M.U. ; on the 104th day, 41,000 ; and by the 187th day, it amounted to over 160,000 M.U. per litre, although a considerable amount of variation has been encountered amongst different mares pregnant for the same length of time. After parturition the amount falls rapidly. From a level of 166,600 M.U. just prior to foaling, it dropped, in one of our mares, to 800 units by the thirty-fifth hour, while sixty-one hours after foaling less than 300 units could be demonstrated.

Earliest appearance of Œstrus-Inducing Hormone in Urine.

Specimens of urine from mares (later shown to be pregnant) taken earlier than one month after service have given uniformly negative results when tested. The lowest concentration that could be demonstrated by the test is 300 M.U., and there is some possibility that lesser amounts than this may have been present, but, as pointed out earlier, much more than this amount may readily be excreted during œstrus by a non-pregnant animal. From the fortieth day onwards until about the sixtieth day the amount present may not greatly exceed what is excreted by the non-pregnant mare, and a second test would be desirable to confirm a positive preliminary diagnosis. A negative diagnosis is, however, often as valuable as a positive one at this stage, since it may still be possible to secure a late service from the stallion. In the great majority of cases it

would appear to be advisable to accept a negative diagnosis during this period as definite evidence that a mare under question was non-pregnant.

From the sixtieth day onwards there should normally be only a small percentage of mares which do not excrete a sufficiency of œstrus-inducing hormone to enable a positive diagnosis being accepted, and with such doubtful cases, a second sample, taken ten to fourteen days later, would yield definite information.

Accuracy of the Test.

Although at the present stage a certain number of refinements of detailed technique would seem desirable, the accuracy obtained is sufficiently encouraging to justify the claim that the test can be utilised to enable the practitioner to make a diagnosis of pregnancy earlier than by clinical means.

A total of 390 samples have been examined at the date of writing. In three cases the biological diagnosis did not agree with the clinical evidence. Two of these were among the first few samples to be examined, and were not based upon a quantitative estimation of the hormone content, but solely upon the occurrence of cornification. The explanation of the other erroneous diagnosis seems doubtful. A positive diagnosis was given, but the mare did not prove to be pregnant. There was some suspicion that abortion took place while the mare was at grass during the summer, but the evidence was inconclusive. There is always a possibility that mares with cystic ovaries or those suffering from nymphomania might yield positive results though non-pregnant, and we have some evidence that this has actually happened. The presence of ovarian tumours capable of disturbing endocrine functioning might occasion error in the interpretation of the test, as might the exhibition of an exceedingly intense œstrus at the time of taking the sample. Pseudo-pregnancy, exceedingly rarely recognised in the mare, though common in the bitch and other smaller animals, might account for some cases erroneously diagnosed as pregnant. In the case of the human Pregnancy Diagnosis Station, the test has agreed with clinical findings in over 97 per cent. of cases, and nowadays the scientific interest is concentrated upon those cases in which disagreement exists. This, undoubtedly, will be the case also if and when the routine pregnancy diagnosis is extended to the cases of domesticated animals.

An Equine Pregnancy Diagnosis Station (?)

It is our intention, should a sufficient amount of support be forthcoming from those engaged in, or those in contact with, horse-

breeding, to establish a pregnancy disagnosis station for the larger animals on the lines of the human station of which mention has been made.

It should be understood that such a development would mark the transformation of what is now a purely investigational activity into an organised, self-supporting mechanism, dedicated to the service of the veterinary profession. No one would say that monies meant for research should be spent for diagnostic purposes, and therefore we await the decision of the profession at large. If it should be that interested veterinary practitioners require the services of this equine pregnancy diagnosis station, then we will arrange it. Of course, money will be required. We might seek it by broadcasting an appeal, but this cannot be hopeful. It would be much better, in our opinion, if we could enlist the interest of a sufficient number of veterinary practitioners who would ensure the maintenance of this station during its formative period by sending in a sufficiency of samples to be tested, and if, for the test, a charge could be made. Obviously, the amount to be charged would be determined mainly by the numbers of samples sent in ; the more, the cheaper. The problem of running the station is a simple one. It is that of making the charge of the test less than 10s., and at the same time securing a revenue of not less than £200 for the provision of a salary of a technical assistant, cost of mice and their maintenance, and postages. Those of us who have been involved in this work and have seen its development, are of the opinion that it offers something of considerable value to veterinary medicine. With refinements, there can be no doubt that the test will become a routine practice sooner or later. Too often we hear of the lag between scientific discovery and its application to practical affairs. If in the case of pregnancy diagnosis there is this lag, the blame must rest with those members of the profession who read this article and disregard its import.

Acknowledgments.

We desire to offer our thanks to those members of the profession in this and other countries who have supplied us with the samples which have provided us with the material required during the past year's investigations. To them and to others we look for a continuance and extension of support for the future.

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THE CO-ORDINATION OF THE REPRODUCTIVE PROCESSES.

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THE success of the live-stock breeder is so closely dependent upon the reproductive powers of his stock that no excuse is needed for considering the physiological factors concerned in the regulation of the reproductive processes. In this article it is hoped to show how our increasing knowledge of the physiology of reproduction bears on the problems of fertility, and to what extent physiological weapons may in future be added to the armoury of the veterinary practitioner called upon to deal with abnormalities arising from ovarian deficiency.

The production of young is the result of a long chain of delicately synchronised and co-ordinated events. A flaw at any stage results in the breakdown of the process, and causes complete or partial inability to bear young. Roughly speaking, the events necessary to bring healthy young to the time when they become independent of the mother are: (*a*) production of ova by the ovary; (*b*) fertilisation; (*c*) implantation of the fertilised ovum; (*d*) retention of the fœtus by the mother until (*e*) parturition, followed by (*f*) adequate lactation.

This article is not concerned with the physiology of the male, or with the mechanism of fertilisation. The remaining events listed above are regulated almost exclusively by means of chemical substances elaborated in and secreted by one organ of the body, and carried by the circulating blood to another organ, which is thus caused to undergo development or other change. These substances belong to the class known as hormones, of which the secretion of the thyroid gland is probably the best known. The inter-relation of the hormones associated with the reproductive processes is extraordinarily complex, and presents some of the most difficult problems so far found in the study of the chemical co-ordination of the animal body.

The ovarian cycle may be divided into two main phases: (*a*) the maturation of the Gräafian follicle culminating in ovulation; and (*b*) the subsequent development of the corpus luteum from the shell of the ruptured follicle. These are respectively known as the "follicular phase" and the "luteal phase." In each phase characteristic

changes take place in the accessory organs of reproduction, vagina, uterus, and mammary glands. In the follicular phase the most obvious changes are in the vagina, and are designed to facilitate copulation, which in all known mammals, except Primates and the rabbit, is restricted to this time, the so-called period of " heat " or œstrus. Thus in the cow a profuse secretion of mucus is found, in the ferret enlargement of the vulva, and in the mouse keratinisation of the epithelium, etc.

During the luteal phase, associated with the development of the corpus luteum, marked changes take place in the uterine mucosa. The essential change, found in all known animals, is an increased sensitivity to mechanical or other irritation ; in many animals, such as the rabbit, this is accompanied by great development of the glands of the mucosa. The post-ovulation changes in the uterus have one very definite and important function, *i.e.*, the facilitation of the implantation of the fertilised ovum. In the absence of these changes implantation does not occur.

Much of our knowledge of the ovarian cycle has been determined from the study of animals in which pregnancy is avoided, either by sterilising the male or by preventing copulation altogether. In the rabbit and ferret copulation is necessary to cause ovulation, but in all farm animals ovulation appears to take place automatically at the time of " heat." In the mouse and rat, ovulation is also spontaneous, but the pre-gestational changes in the uterus are only found after copulation. When pregnancy fails to occur the prepared uterus, after a so-called period of pseudo-pregnancy, sooner or later undergoes retrogression, and the reproductive organs either revert to a quiescent state of anœstrus or else prepare for another period of œstrus and ovulation. The dog, for instance, only has one ovulation in a breeding season, while the unmated mouse ovulates regularly every five days. Farm animals have at least a short series of œstrous periods if pregnancy is delayed. The non-pregnant cow, for example, comes into " heat " about every three weeks during the greater part of the year. During pregnancy ovulation does not occur ; during lactation events vary greatly from species to species. The ferret, for instance, does not come into " heat " while suckling young ; in the cow, on the other hand, the cycle starts again soon after parturition, and this animal normally becomes pregnant again during lactation.

It is now necessary to consider the means whereby the changes in the accessory organs are synchronised with the corresponding events in the ovaries. Removal of the ovaries, of course, stops

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all cyclic activity in the uterus and vagina, while removal of the accessory organs does not affect the ovarian cycle. It is thus evident that the ovary is responsible for the cycle in the accessory organs. As regards the follicular phase, it is now known that the changes in the vagina and uterus at the time of " heat " are due to the action of a hormone secreted by the ovary at this time, and having a specific action on the female reproductive tract. This hormone has now been isolated in crystalline form and has been studied in considerable detail.

The injection of this substance into the ovariectomised animal leads to the appearance of all the extra-ovarian symptoms of œstrus, including willingness to copulate. Comparatively small amounts have a remarkable effect ; thus the typical cornification of the vagina of the mouse may be induced by the injection of as little as one eight-millionth of a gram of the pure hormone. It is evident, therefore, that a very powerful substance is now available, and considerable use will possibly be found for it in veterinary practice. *A priori* one might expect that it would be of value in cases where ovarian deficiency leads to the non-appearance or ill-development of " heat," but as the substance appears to have little or no action on the ovary itself, its use is obviously limited.

As regards the luteal phase, the situation is more complicated. The post-ovulation events are largely controlled by the corpus luteum, and a mass of experimental work has made it obvious that three functions may be attributed to this body : (*a*) Control of the pre-gestational changes in the uterus ; (*b*) maintenance of pregnancy ; (*a*) control of the mammary glands during at least the first part of their development.

Research has also established the fact that these functions are carried out by means of one or more chemical substances secreted by the corpus luteum. An extract of luteal tissue has recently been prepared which will induce the pre-gestational development of the uterine endometrium and also maintain pregnancy in the ovarectomised animal. Since the extract is comparatively crude at the moment, it is not yet evident whether one hormone is responsible for both these actions. So far, mammary development similar to that found during pregnancy does not appear to have been induced by corpus luteum extracts. Superficially it would appear that clinical scope will be found for the luteal hormones in cases where the activity of the corpus luteum is deficient, but, as with the œstrus-producing substance, the action is limited to the accessory organs, and the primary ovarian deficiency is untouched.

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From both a theoretical and practical standpoint the critical mechanism is that controlling the activity of the ovary itself, and very recently discoveries have been made which are likely to be of paramount importance in medical and veterinary practice. In each of its two main phases of activity, the ovary undergoes certain morphological changes, and coincidently produces certain hormones to stimulate the accessory organs. For many years evidence has existed that the mechanism controlling both the morphological and the endocrine activities is situated outside the ovary itself, and recently it has been found that the anterior lobe of the pituitary body produces one or more substances which govern the ovarian cycle and thence the cycle in the accessory organs. Thus, the injection into the immature animal of one type of extract of the anterior pituitary lobe results in the rapid maturation of the immature follicles, and the consequent appearance of œstrous changes in the uterus and vagina. Another type of extract causes the wholesale luteinisation of the follicular granulosa, and the appearance of large numbers of functional corpora lutea. It is not yet clear whether two separate pituitary substances are involved in causing these reactions, but it is evident that the anterior lobe is capable of inducing both the main phases of ovarian activity, and is almost certainly responsible for their regulation in the normal animal. Conversely, abnormalities of the reproductive processes, in so far as they are due to ovarian excess or deficiency, presumably have their origin in dysfunction of the pituitary body.

The pituitary substances have not yet been prepared in any great degree of purification, and the investigation of their physiological effects is still in its infancy, but sooner or later they will be available in a pure form with an accurately known physiological action. When this has been achieved, the experimental physiologist will have placed a weapon of unsurpassed potency in the hands of both the medical and veterinary gynæcologist.

THE REGULATION OF THE ACID-BASE BALANCE OF THE BODY.

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THE co-ordination of the respiratory and buffer functions of the blood with the activities of the organism affords a most instructive example of the unitary aspect of a living being. That a more or

less complete picture of this functional interplay can be presented is due to the labours of many physiologists who have attacked, during the last fifteen to twenty years, the problems involved; but although this work has been most extensively carried out on the human subject, yet domesticated animals offer many suggestions for similar investigations, and exhibit phenomena which must modify the results. The following outline of the subject gives some of the more salient points which may afford an introduction to further study.

The Physico-Chemical Properties of the Blood considered as an Isolated System.

The plasma of the blood separated from the cells present phenomena which characterise watery solutions containing mixtures of substances termed "buffer systems." This term implies that acids or alkalies may be added in considerable amounts to such solutions, with slight alteration of the acid or alkaline condition originally present. Had the acids or alkalies been added to pure water, an immediate and marked change would have been observed.

In order to understand this difference in behaviour, it is necessary to refer to some of the properties displayed by these systems. The characteristics common to all acids are considered to be due to a greater or less proportion of the molecules present dissociating or splitting into two parts, so that a solution of an acid may be visualised as containing at least three types of bodies. For example, in carbonic acid (H_2CO_3) a certain percentage of the molecules dissociate into the electrically active ions, H and HCO_3. Thus H_2CO_3 molecules, H ions and HCO_3 ions are present. The acid properties of the solution are due to the hydrogen ions. In the case of this acid the percentage of acid molecules so dissociating is small. Such acids are termed weak, while acids such as hydrochloric, where a greater proportion of the molecules dissociate, are termed strong acids.

Alkalies, on the other hand, give rise to OH ions (hydroxyl ions) and the proportion of molecules which dissociate confer a corresponding degree of alkalinity on the solution. For example, NaOH dissociates into Na and OH ions. The *concentration* of H and OH ions, *viz.*, the amounts in fractions of a gramme present in the volume of one litre of solution, may be expressed by the symbols cH. and cOH.

Water dissociates to a very slight extent into H and OH ($H_2O \gtrless H+OH$), therefore in all watery solutions, whether acid or alkaline, both ions are present. In all solutions the product of these

concentrations is always constant ($cH \times cOH = a$ constant), therefore the addition of an acid or of an alkali producing an increase of H ions in the one case and of OH ions in the other, involves a corresponding decrease of OH with an acid addition and of H with an alkali addition in order to maintain the constancy of the product of the concentrations.

As already stated, considerable quantities of acids or alkalies may be added to blood plasma without producing alterations in acidity and alkalinity, which would occur if the addition had been made to pure water. This buffer phenomenon is principally due to the simultaneous presence of dissolved carbon dioxide (carbonic acid) and of its salt, sodium bicarbonate ($NaHCO_3$). A weak acid such as carbonic, together with its salt of a strong base, *e.g.*, sodium, is an example of a buffer system. If a stronger acid, such as hydrochloric, is added to such a system, then the stronger acid obtains possession of the base, forming in this case NaCl, and setting free H_2CO_3 from the bicarbonate. This H_2CO_3 being a weak acid, gives rise to fewer hydrogen ions than the corresponding number of molecules of HCl acting alone. Further, the presence of an excess of bicarbonate depresses or prevents the H_2CO_3 dissociating to the same degree that it would if it only were present. These two events, *i.e.*, the replacement of the stronger acid by the weaker, and the depression of the dissociation of the weaker by its salt, results in a much less change in acidity than would otherwise occur.

In the case of blood plasma, this property is termed the *primary buffering* of the blood. It is for the greater part due to this bicarbonate system. Phosphates and proteins also contribute, but to a much lesser degree. Whole blood, in addition to this primary buffering, possesses further buffer properties in virtue of the presence of the red cells. The effect of their presence is as follows: When the concentration of dissolved carbon dioxide (carbonic acid) increases, an interaction occurs with the chlorides of the plasma ($H_2CO_3 + NaCl \gtrless NaHCO_3 + HCl$), whereby the carbonic acid obtains base to form bicarbonate, and the minute quantities of hydrochloric acid arising in the process pass into the red cells. Within these the hæmoglobin, which functions as a weak acid combined in the case of human blood with potassium (KHb and $KHbO_2$), supplies base to neutralise the HCl. This *secondary* or *cellular buffering* further diminishes the tendency to change in the hydrogen ion concentration.

The buffering properties of the blood may therefore be summarised as follows: (*a*) In plasma, interaction of stronger acids

with bicarbonate produces neutralisation of the stronger, setting free the weaker H_2CO_3, in the presence of an excess of $NaHCO_3$. (*b*) In whole blood, in the plasma: $H_2CO_3 + NaCl \gtrless NaHCO_3 + HCl$, and in the red cells, $HCl + KHb$ or $KHbO_2 \gtrless KCl + HHb$ or $HHbO_2$.

The reversed arrows signify that the interaction may proceed in either direction—from left to right in (*a*) when an acid possessing an influence stronger than H_2CO_3 is added to the plasma; and in (*b*) when the concentration of H_2CO_3 increases. Changes in the reverse direction occur when the stronger acid is removed or the concentration of H_2CO_3 diminished. These changes proceed until new positions of equilibrium are attained.

Turning again to the bicarbonate buffering system, the *reaction* of the fluid in which it is present depends upon the ratio between the concentration of the acid to its salt. This is expressed by a well known equation, $cH = \text{a constant} \times \frac{H_2CO_3 \text{ concentration}}{\text{bicarbonate concentration}}$. In human circulating blood this ratio is normally 1/20.

Certain important points must be noted. The concentration of hydrogen ions (cH) or reaction, depending on the *ratio* between the two components remains constant if these components are increased or diminished in equal proportions. It increases either by a diminution in bicarbonate, or by an increase in H_2CO_3 if the other member of the system remains unaltered, or does not change in proportion. In other words, the acidity increases. Reverse alterations will diminish the cH, that is, the reaction will become more alkaline. The *capacity* of the blood to carry carbon dioxide depends on the amount of base which may be available to form bicarbonate. The bicarbonate also functions as a reserve for the neutralisation of stronger acids which gain an entrance into the circulation, for example, lactic acid during severe muscular exercise. It will readily be understood from the foregoing account that the concentrations of the hydrogen ions of the dissolved carbon dioxide and the bicarbonate are intimately connected with each other. Further physico-chemical complexities of the fluid must now be outlined.

The amount of oxygen in combination with the hæmoglobin of the red cells, corresponding to the pressure of oxygen to which the blood is exposed, is influenced by the concentration of carbonic acid. Increase in the latter diminishes, for a given oxygen pressure, the proportion of oxygenated hæmoglobin (oxyhæmoglobin, HbO_2). On the other hand, for a given carbon dioxide pressure, an increase in the oxygen pressure diminishes the amount of bicarbonate formed, and thus influences the capacity of a given blood to carry carbon-

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dioxide. Each of these two gases, oxygen and carbon dioxide, therefore, have an action on the capacity of the blood to carry the other. The partial removal of the oxygen in the tissues allows the venous blood to hold an increased amount of carbonic acid in bicarbonate combination without an undue increase in hydrogen ion concentration. The degree of oxygenation of the hæmoglobin has therefore to be added to the variables already described, which are woven into a complex web of interacting components. Other factors are also connected with these, but they must be omitted from the present outline.

The Relations of the Blood *in vivo*.

So far we have considered the blood as an isolated system containing within itself certain mutual adjustments. In the intact animal this fluid must be brought into relation with the varying activities of the body. When an animal performs muscular work the muscles produce carbon dioxide and lactic acid, and require amounts of oxygen proportionate to the work. Since the limits compatible with health within which the reaction of the blood may alter are very narrow, these demands of the working muscles must be met, and yet the change in reaction must be slight. Unless the exercise be severe, the lactic acid produced can be largely dealt with at the site of production. The increased carbon dioxide enters the blood, but the simultaneous usage of oxygen allows the venous blood to carry away the increase without any marked change in reaction. This slight change, however, is sufficient to cause a greater ventilation of the lungs. This ventilation, together with more rapid circulation of the blood, suffices to deal with the greater production of CO_2 and the increased demand for oxygen. Should the exercise be of such degree of severity that the lactic acid increases in the circulating blood, the buffering powers of this fluid will prevent too great a change in reaction. The kidneys also will participate by the excretion of a larger proportion of acid bodies. Therefore in the intact organism, in addition to the properties of the blood, there are three mechanisms by which the physico-chemical conditions are controlled: (1) The lungs where the output of carbon dioxide and the intake of oxygen involve changes already described. (2) The kidneys, by the excretion of acids or bases in greater or less proportion. Thus an acid urine would result from an increase in acids other than carbon dioxide in the blood; an alkaline urine from an increase in bases. (3) The tissues. The evidence for interaction between blood and tissues is incomplete. There are, however, experimental facts which show that bases may be mobilised from the tissues, that buffering changes similar to those

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between red cells and plasma may occur, and that lactic acid produced in active muscles may be taken up by the muscles not involved in the movements. Lastly, when non-gaseous acids obtain access to the blood some of the ammonia, which otherwise would be converted into urea, neutralises these acids, with the result that ammonia compounds increase in the urine.

The consequences of a change in the reaction of the blood in the direction of increased acidity are, therefore, increase in pulmonary ventilation, which tends to lower the carbon dioxide pressure in the lungs and increase in acid bodies and ammonia compounds in the urine. In pathological degrees of this condition (acidosis, acidæmia), coma is the penultimate event. A change in the direction of increased alkalinity results in a diminution in pulmonary ventilation in order to produce a compensatory retention of carbon dioxide and in an excretion of alkaline urine, with a diminution or complete disappearance of ammonia compounds. When the blood alkalinity is excessive (alkalosis, alkalæmia), spasmodic contractions of muscles may occur similar to the clinical condition of tetany.

When we turn to the domesticated animal we find that the control of the acid base balance is largely unexplored. It is obvious on *a priori* grounds that the conditions must in many cases be greatly modified, and the interactions, if not differing in kind, must be set at a different level.

Herbivora, with a normal excretion of alkaline urine, may react differently to an acidosis or an alkalosis. The dog, possessing a marked regulation by the lungs of temperature control, may exhibit reactions due to this factor, for it has been shown that hot baths cause in the human subject a tendency to an alkalosis, the pulmonary channel of heat loss being used to compensate for the abolition of cutaneous control, so that an undue loss of carbon dioxide results. Birds might react differently to an acidosis owing to the replacement of urea by uric acid in their urine.

The above sketch of the main events of acid-base equilibrium in the human subject is necessarily incomplete. It has been written with the intention of arousing the interest of those who have access to the necessary material, and of forming a starting point for further study.

Detailed expositions of the theory of the regulation of the acid-base balance of the body, and the methods of investigation, with references to the literature, may be found in : " Recent Advances in Physiology," by C. Lovatt Evans, 4th ed., London, 1930. van Slyke : " Physiological Reviews," Vol. I, 1921. " Human Experimental Physiology," by F. W. Lamb, 1930, Chapters XIII, XIV and XV.

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STORES OF BLOOD.

By J. BARCROFT, F.R.S.

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THE conception that certain organs can play the rôle of storehouses or depôts for blood, has attained considerable prominence during the last few years. It is desirable to review the experimental evidence on which this claim is based. The organs in question are the spleen, the liver, and the papillary vessels of the skin. To these may perhaps be added the veins of the pregnant uterus.

The Spleen.

The methods of approach to the question of whether the spleen stores red corpuscles and expels them into the system when such are required are several, but they resolve themselves into two general types, the indirect and the direct. The indirect method used by Scheunert and Krzywanek (1926), by Binet and his school (1926), and by others, is to compare the reaction of the animal before and after splenectomy. Personally, I have a predilection for direct methods, and therefore I have approached the subject in a no more elaborate way than that of observing the size of the spleen when the organism reacts. The simplest way of making such observations is to exteriorise (Barcroft and Stephens, 1929) the spleen, in which case so marked are the alterations in volume of the organ, that the most casual observation cannot fail to discover considerable alterations in its size. From such alterations may be inferred the expulsion of the stored content of corpuscles. The spleen of a dog of about 18 kilos is capable of holding at least 300 c.c. of blood, *i.e.*, roughly 2,000,000,000,000 red corpuscles, which can be expelled in case of necessity into the circulation. The number of corpuscles in the dog would be about 9,000,000,000,000, so that approximately one-fifth of the corpuscles of the dog may be held in reserve in the spleen. The occasions on which the spleen vents itself may be classified as those in which there is immediate need either for a blood richer in corpuscles, or for an increased blood volume. The line between the two is probably not a very hard and fast one. The reaction of the spleen to exercise has been discussed in the papers referred to above; here I will mention some other acute conditions in which the spleen gives up its blood, and also some less acute, but not less interesting ones. Acute conditions, other than exercise, are hæmorrhage, oxygen want, mental agitation, and the influence of anæsthetics. Subacute ones are pregnancy, post-operative conditions, and possibly lactation.

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(1) *Hæmorrhage.*—When an animal is bled, the spleen contracts, and it is possible in a crude way to measure the volume shrinkage. Hence a comparison may be instituted between the amount of blood taken out of store, or at least out of that particular store, and the amount of blood lost. Such a comparison reveals the fact that though in the early stages of hæmorrhage the blood volume scarcely diminishes at all, the spleen making up immediately almost what the animal loses as the result of the hæmorrhage. The following figures were obtained from a dog :

SUCCESSIVE BLEEDINGS.

A = Total quantity of blood lost by animal
B = Total quantity of blood lost by spleen.

	(1)	(2)	(3)	(4)	(5)	(6)	
A	20	40	68	133	200	230	c.c.
B	5	25	39	44	50	52	c.c.

And also from a cat :

A	10	37	61	
B	9.8	16.5	20.2	

(2) *Emotional Conditions.*—The effects of emotion on the spleen were first demonstrated by Hargis and Mann (1925), who constructed a type of plethysmograph in which the spleen could lie without apparent discomfort to the fully conscious animal. Events such as the closing of the door, or any sudden noise, caused contraction of the organ. Izquierdo and Cannon (1928) have pointed out that such conditions as fear and rage, those in fact in which adrenalin is excreted, produce a marked increase in the red blood corpuscle count in cats which contain spleens, but on the other hand, in splenectomised cats no such increase takes place. Previously to their experiments, Binet and his pupils observed that adrenalin itself raises the red blood count in animals which contain spleens, but not in animals from which those organs have been removed. Feldberg has correlated the observed contraction of the spleen with the increase in the red blood count. So far as my own observations go, the emotional conditions which I have studied have been such as precede exercise, or are calculated to precede it. In this connection an attempt (Barcroft, 1930) was made to grade the emotional stimuli, and to discover what grade of stimulus evoked the response (*a*) of the spleen, and (*b*) of voluntary muscle. These experiments were carried out on a dog in which the desire to pursue any cat in the vicinity was rather, but not too strongly, marked.

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(*a*) If this dog were laid on the table and a clean duster dangled in front of its nose, no notice was taken of it, and the size of the spleen remained unaltered. If for that duster another was substituted which had been in a basket where there was a cat, there was an appreciable though not large contraction of the spleen. There was no gross muscular response in the sense of effort to move. It is not possible to say whether there was heightened tone of the skeletal muscles (Fig. 2).

(*b*) If the cat, being in an adjacent room, were caused to " mew," a further but still incomplete contraction of the spleen would take place (Fig. 2). The muscular movement associated was at most pricking up of the ears, and movements of the eyes and head.

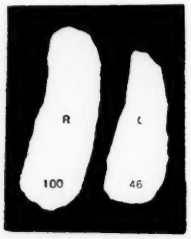

FIG. 1

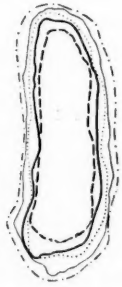

FIG. 2

FIG. 1.—R=rest; C=dog sees cat. The numbers represent the relative sizes of the cat's spleen.

FIG. 2. —.— rest; —— smells cat; ····· hears cat; —— sees cat; - - - - - chases cat.

(*c*) The cat was then brought into the room and placed where the dog could see it, and about two feet away. This procedure was associated with a further contraction of the spleen and muscular restlessness on the part of the dog (Fig. 2).

(*d*) Finally the dog was told to chase the cat, which it was allowed to do for about a quarter of a minute, with the result that its spleen contracted still more. In one case, that of June 5th, the contraction was probably almost maximal, at all events the surface of the spleen (13.5 sq. cm.) approached closely to that in the animal post-mortem (13 sq. cm.). This dog was killed on October 30th following. The following table gives a numerical statement of the effects observed (*see also* Fig. 1).

APPROXIMATE VOLUME OF DOG'S SPLEEN EXPRESSED AS A PERCENTAGE OF THE VOLUME AT REST BEFORE THE EXPERIMENT.

Date	Rest	Smells Cat	Hears Cat	Sees Cat	Chases Cat
March 29th	100	—	64	—	26
April 2nd	100	—	—	45	28
April 3rd	100	—	—	46	30
April 7th	100	—	—	34	25
May 4th	100	72	51	39	—
June 5th	100	62	54	33	13

The figures suggest a better response on the part of the spleen at some times than at others. The measurements on which they are based are, however, too rough for any stress to be laid on this point.

If no more than the emotions were involved there would be little point in the expulsion of a considerable quantity of blood from the store into the circulation. The significance appears to be that the series of emotions enumerated ultimately lead up to an effort of physical exercise, and that the spleen has anticipated the demand for that increase in the blood volume which is part and parcel of the complex which insures augmentation of the minute volume of circulating blood.

(3) *Carbon Monoxide.*—The original discovery that the spleen acted as a store of blood depended upon the fact that carbon monoxide given in doses insufficient to upset the circulation only reaches the hæmoglobin of the spleen pulp very slowly (Barcroft, J. and H., 1923). But if the gas be administered in greater concentrations it produces a contraction of the spleen with consequent expulsion of the splenic contents (Hanak and Harkavy, 1925). The mechanism of this contraction was studied by Carrol and de Boer (1924). They showed that if the spleen was connected with the body solely by the splenic nerve, the spleen itself being perfused from an artificial circulation, the administration of CO to the animal (none of whose blood entered the spleen) produced contractions of the organ, whilst the conversion of a considerable portion of the hæmaglobin in the blood which traversed the spleen did not affect the volume of the organ. The effect of the carbon monoxide is on the nervous system, and it can act effectively on the spinal cord. The expulsion of blood from the spleen on administration of CO furnishes an example of the fact that the possession of a store of blood may under certain chosen circumstances determine the difference between life and death.

Three groups of guinea-pigs were exposed to carbon monoxide in lethal doses. Group I, normal; Group II, splenectomised; Group III had been subjected to an abdominal operation which did not involve the spleen. Of the three groups, the second died in the carbon monoxide chamber before the members of Groups I and III; had

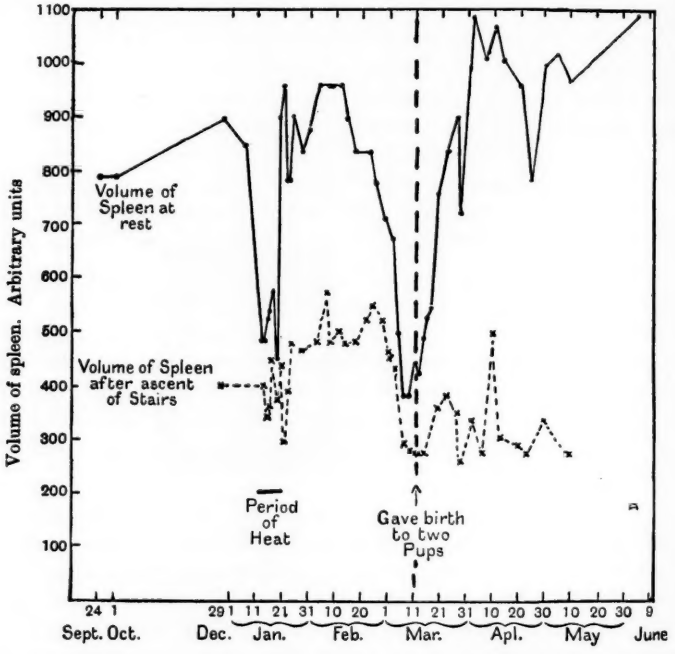

FIG. 3.—Computed volume of spleen: ·–·–·– at rest and after standard exercise. × – – × – – × during heat and pregnancy. ⍝ after chasing cat along passage. The mass of the spleen in grams is not very different from the figure obtained by dividing the arbitrary units on the ordinate by fifteen.

[We are indebted to the *Journal of Physiology*, Vol. 66, p. 35, for permission to use this illustration.]

these been removed from the chamber just after Group II died, they (*i.e.* I and III) would have survived, and would have owed their survival to their spleens.

(4) *Certain Anæsthetics.*—No phenomenon in connection with the volume of the spleen is more striking than the shrinking of this organ when chloroform or ether are administered either separately or together. The smell of these anæsthetics will cause contraction of the spleen in many dogs; that, however, is clearly a different phenomenon, and

in so far as it is of interest, it should have been mentioned among the reactions of the spleen to emotion. On the administration of anæsthetic doses, the spleen usually contracts to a very small bulk, expelling its contents into the circulation. It may be of some comfort to the surgeon to reflect that when he is operating (certainly this is so in the dog, cat, etc.) there is an abnormally large quantity of blood in the vessels.

Alcohol produces no such reaction: the only shrinkage of the exteriorised spleen which I have observed in dogs under the influence of alcohol seemed to me to be associated entirely with general excite-

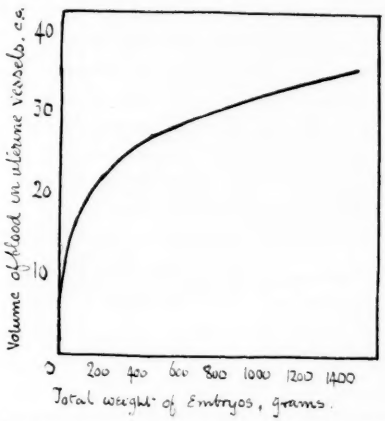

FIG. 4.—Relation of quantity of blood in uterine vessels of bitch to tota weight of contained embryos. Probably correct to within 25%.

ment. During the condition of complete unconsciousness there is not in the case of alcohol, as there is in the case of ether, a contracted spleen. Interesting as are the conditions which evoke passing contraction of the spleen, the causes of long-continued contraction are not less interesting. The two best established of these are pregnancy (and heat) (Barcroft and Stevens, 1928), and certain post-operative conditions.

(1) *Pregnancy.*—Concerning pregnancy, it is evident that in the later stages there is a quite unusual volume of blood in the generative organs. It is not very easy to measure the blood in the generative organs or to correlate with any other variable. Paul Rothschild and I (1931) have made determinations on rabbits, cats, and dogs, in which animals the most obvious correlation has been that between the quantity of blood in the uterus and the total

weight attained by the fœtuses. Here it need only be said that in all these types, in spite of this difference in the size of the adult animal, the amount of blood in the generative organs is commonly in the neighbourhood of 30–35 c.c. at the end of pregnancy, and after parturition the quantity rapidly diminishes to something negligible. This quantity of blood in the case of the dog at all events seems to be transferred from the spleen—a very intelligible arrangement, when it is recalled that from the metabolic point of view the biochemical situation in the mother is no doubt being exploited for the provision of blood in the rapidly growing embryos. Thus the fœtuses within the uterus of a bitch of 18 kilos may weigh over two kilos, and may contain perhaps 200 c.c. of blood. This must be made, most of it, within a month, from materials supplied by the mother. It is not surprising, therefore, that the mechanical needs of the hypertrophied maternal organs should be met from the store in the spleen. The contraction of the spleen in the dog is usually well-marked during heat, when the generative organs become turgid ; when heat passes off, it resumes its normal size, and contraction sets in again about half-way through pregnancy, coming on gradually, reaching its most accentuated condition about two days before the pups are born.

A word here concerning the mechanism of this pregnancy contraction : is the transference of blood from the spleen to the uterus the result of a hormone, or must it be attributed to the action of the nervous system ? So far as our meagre knowledge goes, the latter is correct. A bitch, the spleen of which had been carefully denervated, has been observed now through two pregnancies. Neither at heat nor during the pregnancy was there any marked contraction of the spleen. So far as we know, therefore, the pregnancy contraction of the spleen is a manifestation of the activity of the nervous system. The reaction noticed in this animal was a rather curious one, namely, that the spleen contracted, not during, but after pregnancy ; whether it was a coincidence or not, the post-partum contraction corresponded with the period of suckling of the young. The phenomenon was clearly observed after each of the pregnancies ; in the second case the time of return of the spleen to its normal size was watched, and it commenced before the pups were removed, but coincidently with these commencing to feed from their mother's dish.

Of the instances of splenic contraction which persist over weeks, none is more striking than that following operative procedure. The number of recorded cases is not large, but they are very remarkable. During the course of experiments, in which parts of the intestine were exteriorised, some time after exteriorisation of the

spleen, observation revealed some quite unexpected facts. In the first two experiments of the series a loop of small intestine was withdrawn and placed outside the abdominal wall. In both cases the animal seemed to do well, even though the gut had not been severed, and the food had to pass along the exteriorised portion. Neither dog, however, lived over four weeks; the ultimate trouble in each case being perforation. In one animal the lesion was in the abdominal cavity. The dog became suddenly ill; the condition clearly being hopeless, it was killed. The post-mortem examination showed a general infection of the abdomen.

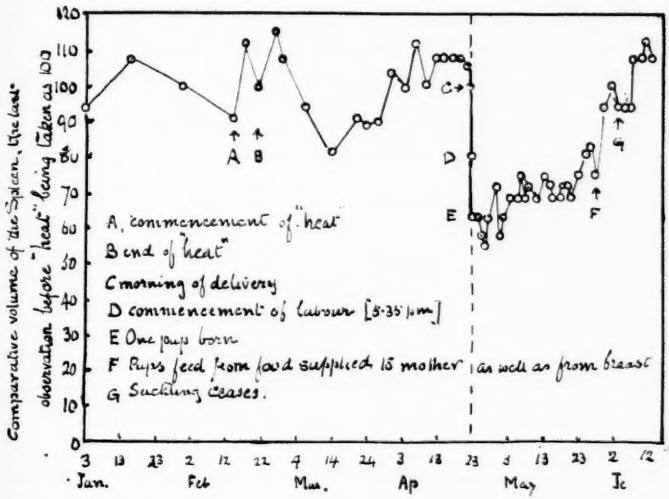
Comparative volume of the Spleen, the last observation before "heat" being taken as 100
A commencement of "heat"
B end of "heat"
C morning of delivery
D commencement of labour [5·35 p.m.]
E One pup born
F Pups feed from food supplied by mother as well as from breast
G Sucking ceases.
Jan.
Feb
Mar.
Ap
May
Je

FIG. 5.—Computed volume of denervated spleen during heat and pregnancy.

The perforation in the other animal took place in the exteriorised portion; it was due to an accidental cause, namely, that the dog swallowed a piece of bone about four inches long. This was unable to get round an angle where the gut was fixed to the abdominal wall. In both these cases the spleen became quite small during the operation, and did not regain the size thereafter. Indeed, in the first case, that in which general peritonitis developed, the spleen shrunk even more than in the latter, in which only perforation took place; it was at its smallest when the animal died. The question arose: Had these animals survived, would their spleens ever have attained to a size as great as that which they would have occupied if the subsequent abdominal operation had never been performed?

One experiment has taken place in which a portion of gut has been exteriorised with perfect success by Florey (1929), after the spleen had been exteriorised some two months previously. There was no indication of any general or even local infection. Nevertheless, the spleen shrank immediately on the intestinal operation to about half the previous size, and remained so for about four weeks. After that time it dilated, and attained the size which, judging from a control dog, it would have occupied if no intestinal operation had taken place. Here, again, we are in the dark as to whether the contraction was due to a nervous reflex or to some substance absorbed from the wound or elsewhere. A less severe operation is that of opening the abdomen, exposing the intestines under clean conditions, pulling them back and sewing up the abdominal wall. Even this produces a contraction of the spleen which lasts for some days. The following are approximately the relative volumes of the spleen in an animal in which the operation was performed on the 140th day after the exteriorisation, as shown in Fig. 6 of the organ :

Day after Exteriorisation of spleen.	Approximate relative volume of spleen.	Remarks.	Temp. ° F.
116	108	—	—
123	93	—	—
140	100	Before operation	—
	66	After operation	—
142	69	—	100
144	66	—	100
148	83	—	100
149	—	—	98
152	88	—	—

The wound cleared up excellently.

The experiments described so far have all been abdominal. Let us pass to a different type of operation, namely, excision of the superior cervical ganglion. Probably owing to my own ineptness, this has always proved a long operation, and one which has involved a good deal of manipulation to the tissues, the ganglion being deeply seated, and care being necessary to insure the completeness of the operation ; but, one the other hand, it does not involve the opening of any large cavity such as the abdomen. There is more to be expected from the results of absorption of injured tissue, and less from general sepsis, than when the abdomen is opened. The results on the volume of the

spleen of the two operations are shown in Fig. 7. Except in the duration of the after-effects, there appears to be little to choose.

Indeed, to obtain a traumatic contraction of the spleen it is not necessary to open the body at all. A very marked shrinkage was obtained unexpectedly in one animal: examination proved that underneath the collar there was a considerable abrasion; the spleen remained somewhat shrunken till the skin wound dried up.

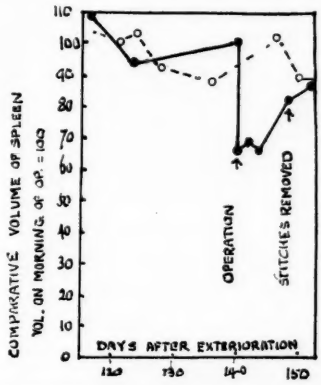

FIG. 6.—Effect of opening of the abdomen on the volume of the spleen.

The Uterus.

In our discussion of the spleen, it was shown how in the dog, blood was apparently transferred from that organ to the uterus during pregnancy. That is not the whole story, in fact it is but one chapter, and at that, a chapter rather near the end of the book. In the first place the spleen appears to be unessential to the presence of large quantities of blood in the uterine vessels. This is well shown in the rabbit, in which animal the uterine blood forms a much greater percentage of the whole blood of the animal than it does in the dog. When the fœtuses are approaching "full time" the dog and the rabbit possess about the same volume of blood in the uterine vessels, namely, 30 ± 5 c.c., and this though the dog may be half a dozen times as heavy as the rabbit, and presumably may contain half a dozen times as much blood.

In the second place, this blood for the most part is not in the uterus itself, but in the veins which lead therefrom. In the rabbit the development of these veins is very remarkable. There are five main vessels; the ovarian veins and the vaginal as in the dog,

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and in addition two great vessels which run into the femoral veins. These lead from an arcade in the broad ligament, which arcade is connected with the uterus by a great number of large veins. The

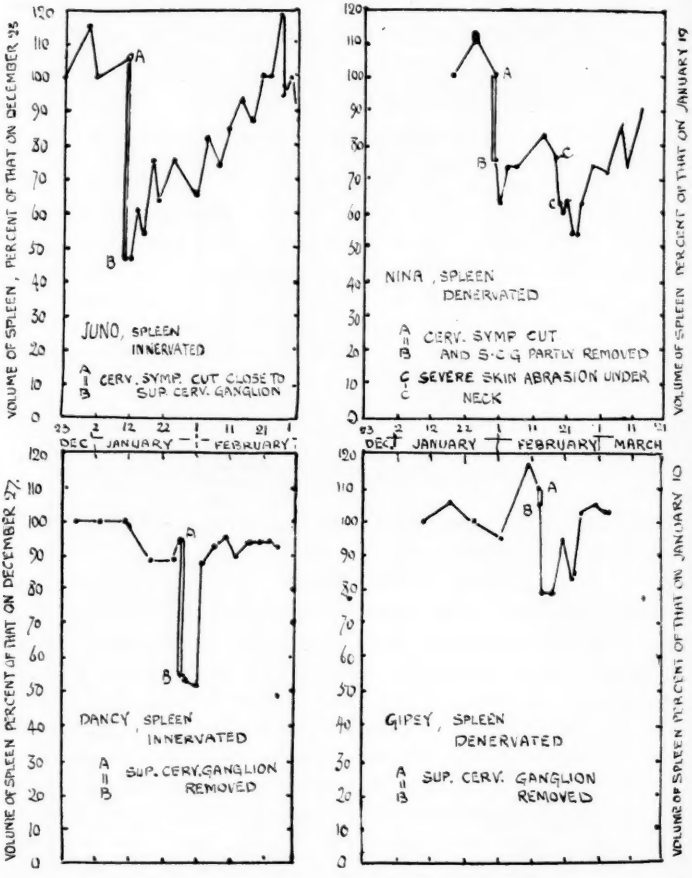
VOLUME OF SPLEEN, PERCENT OF THAT ON DECEMBER 25
JUNO, SPLEEN INNERVATED
A = B CERV. SYMP. CUT CLOSE TO SUP. CERV. GANGLION
NINA, SPLEEN DENERVATED
A = B CERV. SYMP. CUT AND S.C.G. PARTLY REMOVED
C SEVERE SKIN ABRASION UNDER NECK
VOLUME OF SPLEEN PERCENT OF THAT ON JANUARY 19
DEC. JANUARY FEBRUARY MARCH
VOLUME OF SPLEEN PERCENT OF THAT ON DECEMBER 27
DANCY, SPLEEN INNERVATED
A = B SUP. CERV. GANGLION REMOVED
GIPEY, SPLEEN DENERVATED
A = B SUP. CERV. GANGLION REMOVED
VOLUME OF SPLEEN PERCENT OF THAT ON JANUARY 10

FIG. 7.—Effects of operation on the volume of spleens which have and have not been denervated respectively.

uterus itself does not contain a great deal of blood. It is clear, therefore, that a great part of the blood in the uterine vessels has no obvious connection with the life of the fœtuses.

In the third place, the general relation of the weight of the fœtuses to that of the uterine blood is shown in figures for dogs, cats, and rab-

bits (*see* Fig. 8). The impression gained from a comparison of these curves is that they may be divided into periods: (1) In which the blood is greatly increased, but before the fœtuses have attained any con-

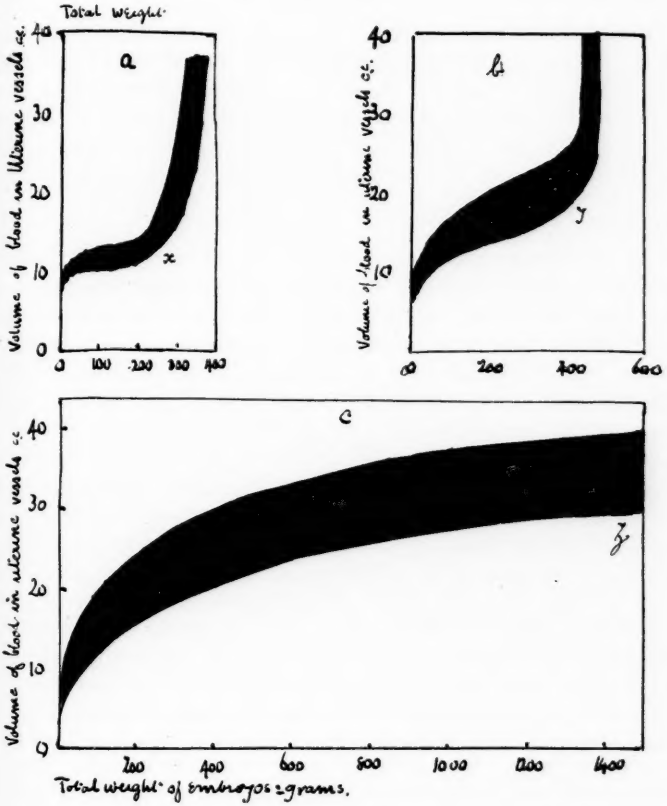

FIG. 8.—Approximate relation between the quantity of blood in the uterine vessels and the total weight of the embryos. *a*=rabbits; *b*=cats; *c*=dogs.

siderable size. (2) In which the fœtuses grow rapidly, the blood content increasing somewhat at the same time. (3) In which in the last days, in the rabbit and to a less extent in the cat, there is a great increase in the blood without a corresponding increase in the fœtal growth. The final stage in the rabbit accounts for more than half the blood, in the cat to perhaps one-quarter to one-third, and in the dog that stage is absent.

The general suggestion which the curves present is that the points *x*, *y*, and *z* respectively on the rabbit, cat, and dog curves, correspond and indicate the proliferation which is consequential to fœtal growth, whilst *aa* and *bb* represent simple storage in the great uterine veins.

The fourth point to be noted is that all excessive blood disappears from the uterus rapidly after birth. Our determination made on the rabbit during delivery showed but 10 c.c. of blood, where previously there had been perhaps 30; our two determinations made on rabbits the day after delivery gave 6 and 9 c.c. respectively. The blood goes elsewhere. What may be the object of this accumulation and subsequent disappearance of blood in the uterine veins we do not as yet know. Two possibilities suggest themselves. Firstly, that it is an insurance against hæmorrhage during confinement; and, secondly, that it provides a supply for the mammary glands when these are called into full activity. The experiments already described on the denervated spleen seem to suggest the latter possibility. The rabbit, which presents this remarkable proliferation of the uterine veins, has a relatively minute spleen.

The Liver.

The quantitative demonstration that the liver acts as a blood depôt has been furnished by Grab, Janssen, and Rein. Their method is one of extreme beauty: the blood-flows in the vessels, going to the liver and coming from it, were measured by the diathermic method, and a balance-sheet was struck of the blood which entered and left the organ. When adrenalin was given the outflow from the liver far exceeded the inflow, so that a large quantity of blood, amounting in one case to over half the weight of the liver itself, was expelled into the general circulation. The following table shows some of Grab, Janssen, and Rein's results:

Blood Expelled from the Liver by Adrenalin.

			Liver Blood.		
No.	Body-weight of dog, k.g.	Liver weight, g.	Balance expelled, c.c.	Per cent. of liver weight.	Adrenalin m.g.
1	11.0	420	250	59	1/50
2	8.0	380	140	37	1/20
3	7.8	300	75	25	1/50
4	12.0	494	130	26	1/100
5	9.0	260	107	41	1/20

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If the blood volume of the dog be taken as one-tenth of its body-weight, the amounts of blood expelled from the liver in the above experiments amount to 23, 18, 10, 11 and 12 per cent. of the blood volume—figures which on the whole appear to be of the same order as are capable of being expelled from the spleen.

The Skin.

Another situation for which a claim has been put forward as a blood depôt, is the sub-papillary venous plexuses of the skin. By way of preface, let me review very briefly the arrangement of the cutaneous vessels. Any description of my own would fall far short of that given by Sir Thomas Lewis: I therefore make no apology for quoting him.

"In his latest account, Spalteholz describes the arteries of the cutaneous arterial network, as possessing a thick muscular coat, which diminishes relatively abruptly about the middle of the corium, where the vessels are clothed by a single layer of muscle cells. Traced further, this layer becomes imperfect, though muscle elements are still discovered on the vessels of the sub-papillary arteriolar plexus, and even on the terminal arterioles. . . . The venous blood returning from the papillæ passes through several networks, the first lies immediately beneath the bases of the papillæ, and receives blood from the venous limbs of the capillary loops and from the minute collecting venules formed by the union of several such capillaries. . . . Almost immediately beneath this is a second venous network, the two intercommunicating freely by short venules; they are often spoken of together as forming the sub-papillary venous plexus. The blood flows deeper by numerous tributaries to the third and fourth venous networks, the former lying immediately deep to the sub-papillary arterial plexus, the latter at the level of the cutaneous plexus of arteries where the cutis and subcutis join. The superficial venous plexuses are formed by endothelium only according to Spalteholz; as the veins are traced more and more deeply, their walls present scattered muscle cells, which become more numerous as the last venous network is reached, and there form an imperfect sheath to the vessels. Valve and full muscular coat first appear in the subcuticular veins. . . .

"Certain deeper and direct communications have been described between arterioles and venules in the bed of the nail and pulp of the fingers and toes by Hoyer and others, and are recognised by Spalteholz. . . . The deep communications are true arterio-venous anastomoses, the arterioles concerned being heavily coated with muscle.

"The broad arrangement of the vessels may be followed from the accompanying diagram (Fig. 9), which is modified from that published by Spalteholz himself. It represents the manner in which

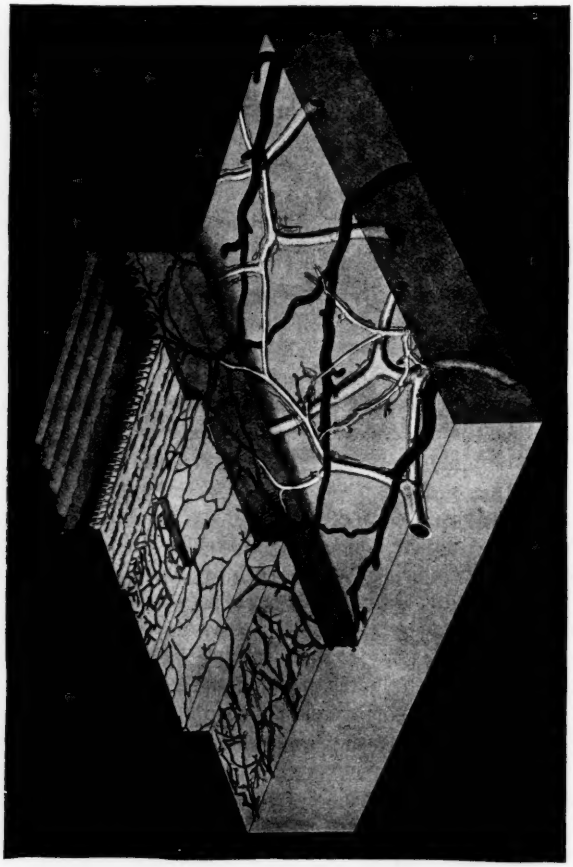

FIG. 9.—A diagram of the skin and its vessels, showing the arrangement of the arterial and venous plexuses at various levels.
[Reproduced with the kind permission of the publishers of "The Blood Vessels of the Human Skin and their Responses," by Sir Thomas Lewis.]

the blood vessels are disposed in the palm of the hand or the sole of the foot, but the same general plan may be recognised in the skin of other parts of the body."

So much for the structure of the skin; now as to the possibility of its acting as a store. The general conception put forward by Dr. Ernst Wollheim (1927) of the Medizinische Universitätsklinik der Charité in Berlin, starts from the observation of Nicolai and

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Jakobi (1920), that the capillaries in the skin of the frog may be divided into two categories: the "stream capillaries," and the "net capillaries." In the stream capillaries there is a continual flow of blood; in the net capillaries, varying quantities of blood can be held for a longer or shorter time side-tracked from the general circulation. In the human fœtus and at birth there are either no, or scarcely any, skin papillæ. In the first months of post-natal life, the capillary networks underneath the skin papillæ rise from the general network of vessels.

In fully developed skin, according to Wollheim, pallor corresponds to a condition in which the narrow end capillaries of the subpapillary plexus are constricted, and only occasionally one sees a peripheral capillary loop emerging from them. Redness signifies a dilatation of the papillary capillaries, and little or no dilatation of the subpapillary plexuses; they are in fact not visible as a complete plexus, but only as occasional vessels.

In cyanotic conditions, on the other hand, the subpapillary networks are dilated: the bluer the cyanosis, the more the subpapillary network comes into the picture as opposed to the papillary capillaries. Wollheim gives no data as to the amount of blood which can be lodged in the subpapillary plexus in normal persons, but he has made numerous observations on cyanotic individuals, of which the following three may be cited: The suggestion is that sitting, the arms and legs are cyanotic, whilst lying the cyanosis clears up, and the blood which, when sitting, was stored in the subpapillary plexuses of the limbs, finds its way into the general circulation. The measurements of the quantity of blood in circulation were made by what in England is known as the "vital red" method of Keith, Geraty, and Rowntree (1915), albeit the actual dye used was trypan blue.

CIRCULATING BLOOD, LYING AND SITTING.

Case.	Body-weight	Volume of circulating plasma litres.	Volume of circulating blood litres.	
(1) Vasoneurosis	50.5	2.7	4.5	Lying: legs and arms pale; subpapillary plexus constricted.
		1.8	3.1	Sitting, one hour later: Legs and arms cyanotic; intense dilatation of subpapillary plexus.

Case.	Body-weight.	Volume of circulating plasma litres.	Volume of circulating blood litres.	
(2) Vasoneurosis with Meniere's Disease	50.3	2.3	4.2	Lying: hands red, arms and face pale-red; plexus normal.
		2.1	3.7	Sitting, 40 minutes later: hands and legs plum-colour; dilated plexus.
(3) Sclero-neurosis	66.5	2.9	5.5	Lying: arms, legs, and countenance dilated capillaries; no visible sub-papillary plexus.
		2.5	5.0	Sitting, eight hours later: legs and hands plum-colour; very dilated sub-papillary plexus.

The above experiments show that, coincidently with the dilatation of the vessels in the subpapillary venous plexus, the blood volume, as measured by the vital red method, decreases by from half a litre to one and a-half litres. The weak point in the experiments is, of course, that they do not exclude the possibility of other blood depôts harbouring a portion of the blood which ceases to circulate, and therefore, they only give a maximal limit in each case for the blood that can have become enclosed in the subpapillary plexuses, not an actual measurement. Taking the lower figure mentioned above, namely, 500 c.c., we may form a sort of estimate of the implication in the following way. The body has a surface of about two square metres, or 20,000 square cm.; were 500 c.c. of blood distributed over this surface as a uniform layer, that layer would be one-quarter of a millimetre in thickness. To me the views of Wollheim have a special interest because they always carry my mind back to the circumstances which originally drew my attention to the possibility of the existence of blood depôts.

On a journey to Peru the blood volume, as measured by the carbon monoxide method, rose when we approached the tropics, reaching a maximum as we passed through the Panama Canal, in three subjects, as follows:

Place.	Blood volume litres.	Total oxygen capacity, litres.	Cabin temp. (about).
MEAKINS—			
36.13 N., 43.53 W.	4.6	0.81	16
Panama	5.9	1.02	26–29
Callao	4.8	0.56	21

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Place.	Blood volume litres.	Total oxygen capacity, litres.	Cabin temp. (about).
BARCROFT—			
Cambridge	4.4	0.83	—
Panama	6.5	1.20	26–29
Callao	4.2	0.84	21
DOGGART—			
Cambridge	4.6	0.96	—
Panama	6.1	1.16	26–29
Callao (near)	5.1	0.96	21

It was possible to check the hypothesis that the apparent alterations in oxygen capacity (*i.e.*, hæmoglobin) and blood volume were associated with the climatic temperature.

On our return to England, Dr. Davies subjected himself to three days' residence in a glass chamber which was heated artificially to about 90° F. (32° C.), with the result that his blood volume rose during the residence and fell again on his return to normal (April) climatic conditions, in Cambridge, associated with east wind, and, it my memory serves me, snow.

	Blood volume litres	Total oxygen capacity	Reticulated cells
DAVIS—			
Before (April 22nd and 23rd)	6.3±0.3	0.98±0.5	0.2
At the end of two days' residence (25th)	7.4±0.4	1.2±0.06	0.9
After (28th)	6.4±0.3	1.0±0.05	0.1

The immediate interest of these figures as regards the skin is that no doubt the circulation in the skin is enormously increased by exposure to heat. It is very improbable that there was any stagnation in the subpapillary plexuses of our skins at Panama, and therefore whatever may be the storage power of the skin, that at least was probably drawn upon forthwith. But I should be far from admitting that in normal individuals under normal winter circumstances (the determinations made on Doggart and myself in Cambridge were in November) a litre of blood was stored in the subpapillary plexuses of the skin. We therefore come to the larger question: What is the storage power of the body?

(To be concluded.)

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THE RETICULO-ENDOTHELIAL SYSTEM.

By A. PINEY, M.D., M.R.C.P.

Director, Pathological Department, Cancer Hospital, London.

THE fate of foreign substances injected into the circulation has interested biologists for centuries, but the number of materials that can be dealt with in this way, without danger, is relatively small. It has often been contended that ordinary histological methods cannot give a true picture of the structure of tissues. For this reason various dye-stuffs have been injected into the living body in the hope that the intact cells could be stained whilst still alive. These methods have not proved particularly successful, because many dye-stuffs are toxic, whilst others are decolourised in the body. There is, nevertheless, a class of substance more or less suitable for this purpose. It must be realised that the nucleus of a cell lying, as it does, in the midst of the cytoplasm, will not be stained by any intra-vital method; this structure is only stainable when dying or dead. Even the protoplasm cannot be stained in the ordinary sense of the word. Injection of suitable substances is followed by the appearance in the cytoplasm of granules consisting of particles of the stain. A variety of substances can be used for this purpose, but two of those most commonly employed are India ink and lithium chromine. Not all the cells of the body exhibit this power of taking up particles of stain; nevertheless, cells containing the granules are found widely scattered, and careful observation has made it possible to prophesy where such stained elements will be found. For example, the large cells which lie in the splenic pulp and line the splenic sinuses will be found to contain particles of the injected dye-stuff. Similarly, the lining of the channels in lymphatic glands, the capillary walls of the bone marrow and the endocytes (Kupffer cells) of the liver possess a similar function. It is obvious that this method does not cause a diffuse staining of the protoplasm such as occurs in ordinary histological preparations. The appearance of particles in the cytoplasm is due to phagocytosis, that is to say, the cells which become stained are those which are capable of ingesting the dye-stuff. Now, as this phagocytic power is common to these widely scattered cells, they are usually regarded as forming a single functional system, the reticulo-endothelium.

It is obvious that the injection of dye-stuffs is a purely experimental procedure, but it is of value inasmuch as it supplies some evidence as to the function of the reticulo-endothelial system. The

recognition of this scattered tissue has led to attempts at investigating its behaviour in normal animals, and it has been found that many important metabolic processes are controlled, at least in part, by this tissue. For example, it is well known that the number of red corpuscles per cmm. of blood remains practically stationary in health, and yet there is a continuous destruction of effete elements and reformation of fresh supplies. The fate of the degenerate red corpuscles and the hæmoglobin that they contain is a problem still awaiting complete solution, but something is already known. The hæmoglobin consists essentially of an iron-containing moiety combined with protein. When red corpuscles are destroyed the hæmoglobin is set free, and the ferruginous fraction is broken down into an iron-free pigment and an iron compound. The former is excreted from the body as bile pigment, whereas the latter is retained for further elaboration into hæmoglobin. In the mammalia the greatest part of the reticulo-endothelium is contained in the spleen, and it is here that the majority of red corpuscles are destroyed. It is even possible to see degenerate remnants of these structures lying inside reticulo-endothelial cells, and it appears certain that it is in the reticulo-endothelium itself that the bile pigment is formed. As the splenic vein carries blood from the spleen to the liver it contains a compound of bilirubin, which undergoes some slight further change in the liver, and is then excreted in the bile. It appears, therefore, that the only source of bile pigment is altered hæmoglobin; and, furthermore, the liver itself is not the essential site of formation of bilirubin, but is more in the nature of an excretory gland. In birds the greater part of the reticulo-endothelium is contained in the liver, and can therefore be removed by excision of this organ. In normal geese, poisoning with arsenuretted hydrogen causes intense jaundice, but if the liver has been removed from the circulation, this phenomenon does not occur. It seems to be proved that the absence of jaundice depends upon the ablation of the reticulo-endothelial cells, and not upon removal of the liver cells themselves.

Many workers have tried to render the reticulo-endothelium inactive by causing its cells to store an excess of dye-stuffs. Large amounts of colloidal dyes have been injected in the hope that the reticulo-endothelium might be " blockaded," and its function thereby markedly reduced or even destroyed. It is, however, not possible to inhibit the reticulo-endothelium completely in this way because its regeneratory power is very great. New cells are formed in large numbers and soon replace the function of the blockaded elements. It is, however, possible to reduce the activity of the tissue, and, when

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this is done, the amount of bile formed in the body is definitely decreased, although the liver cells are still intact. There can be no doubt, therefore, that the reticulo-endothelium is capable of metabolising hæmoglobin into bile pigment, although the liver itself has not this power.

The reticulo-endothelium also plays a part in the metabolism of fat. Even in health globules can be found in many of the cells of this system, but it is only in pathological states that it becomes obvious how important the elements of this tissue must be. In many cases of diabetes there is a marked excess of lipoids in the circulation, and then reticulo-endothelial cells, particularly in the spleen, become much enlarged and increased in number on account of deposition of fat in their protoplasm. As has already been said, in most of the mammalia the main bulk of reticulo-endothelial tissue is found in the spleen, and splenectomy is invariably followed by increase in the amount of cholesterol in the blood. This makes it obvious that the spleen, purely in virtue of its reticulo-endothelial cells, is of great importance in the metabolism of lipoid substances.

Proteins are also dealt with, at any rate to some extent, by the reticulo-endothelium, but it is not so easy to demonstrate this because all cytoplasm contains large amounts of protein. The administration of a diet rich in albuminous material is followed by hyperplasia of the reticulo-endothelium. The individual cells become abnormally large, and there is also increase in number. It is interesting to note that long-continued use of such a diet may cause amyloid degeneration, and it appears, therefore, that all forms of food-stuffs are dealt with by this important and widely scattered tissue.

There can also be little doubt that the cells of the reticulo-endothelial system are the matrix from which all the blood cells are derived. It has already been said that reticulo-endothelium lines the sinuses and is scattered in the pulp of the spleen and lymphatic glands, whilst a similar tissue bounds the lumen of the capillaries in the bone marrow. The function of the reticulo-endothelial cells, like that of all other living units, depends in part upon the structure of the individual elements, and also upon the environment. For this reason the function of the tissue will differ according to its anatomical site; for example, the recticulo-endothelium in lymphatic glands will give rise only to lymphocytes, but, in the totally different environment of the bone marrow, it will produce red corpuscles and granular leucocytes.

Many readers may be puzzled at the name reticulo-endothelium. The term " reticulum " was introduced to designate fine connective

tissue, such as is found in lymphatic glands, bone marrow, liver and spleen. It differs from ordinary fibrous tissue in its chemical composition and in its histological structure. The individual cells are extremely elongated and have branching processes, whilst the nuclei are small. The word " endothelium " has unfortunately been applied to two very different structures. First, the cells lining blood vessels are flattened, more or less square plates, with the nucleus in the middle, and these are endothelium in the strict sense of the word. Secondly, the lining of sinuses in lymphatic glands and of capillaries in the liver and bone marrow has also been called endothelium, but it differs very much from the cells described above. In these channels the wall is incomplete and the cells are not sharply distinguishable from one another, nor are they definitely flattened or square. Even on the aspect facing the lumen there is irregularity due to the great difference in the size of the cells, and, on the inner boundary of the cells no sharp line of demarcation can be detected, the cell apparently being in direct contact with the adjacent reticular tissue. It has even been said that the lining of these channels is incomplete, but this is not quite an accurate statement. The wall consists in part of " endothelium," whereas in other places the reticulum itself is in contact with the contents of the channel. The endothelial lining is not a continuous one, and it is relatively easy for the cells to pass from the tissues into the lumen, or in the opposite direction. Injection of suitable dye-stuffs is followed by the appearance of granules, both in the " endothelial " cells and in the reticular elements ; so that we have now good reason for the name reticulo-endothelium. First, because the two types of cell lie in close juxtaposition one to another ; and, secondly, because they share a common phagocytic function. There is, in addition, sound morphological evidence that the name is justified. Complicated histological methods that depend upon the impregnation of tissues with silver show that the " reticulum " does not only lie close to the " endothelium," but that its fibrils form an integral part of the cytoplasm of the latter structures. Fine argentophilic fibrils can be seen in many of the endothelial cells which never possess a distinct outline. Some of the fibrils appear to end in the cell, whereas others emerge beyond its boundaries, and it is by these latter that the individual endothelial elements are connected with one another, and with the reticular elements. Reticular cells present a similar structure, but the argentophilic fibrils in the cytoplasm run longitudinally, and some emerge from the cell body and form the fine reticulum previously described. It is most important to realise

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that histological preparations such as these show many cells intermediate in type between "endothelium" and "reticulum." There can be no doubt that these two different types of tissue are only different manifestations of one another, and are easily interconvertible according to the needs of the body as a whole.

Only one more reference to the function of this peculiar system is possible here. It has often been supposed that the reticulo-endothelium is the site of formation of antibodies. Many workers have tried to suppress the function of the tissue in order to test this hypothesis, but as has already been said, it is impossible to ablate the reticulo-endothelium. Even so, reduction of the functional activity of the reticulo-endothelium is followed by a decreased power of producing agglutinins. This appears to be sound evidence that at least some anti-substances take their origin from the cells of this system. When the reticulo-endothelium is excessively active in an abnormal manner antibodies are formed in only defective amounts. For example, in chronic myeloid leukæmia, where the reticulo-endothelium is producing numbers of abnormal cells, antibody formation is almost impossible.

The merest outline of modern knowledge concerning the reticulo-endothelium has been given here, but it is hoped that enough has been said to make it clear that this diffused system of cells is one of the most important in the preservation of normal metabolic function. The discovery of internal secretions demonstrated how closely dependent one part of the body might be upon another, even in the absence of nervous connections. The presence of a diffused tissue such as the reticulo-endothelium similarly shows how widely separated are the morphological connections of the body. That there is some relationship between the endocrine glands and the reticulo-endothelial system is extremely probable. Certainly the avidity for storage of foreign substances can be influenced by the administration of various extracts derived from the organs of internal secretion. It is perhaps too much to hope that it will ultimately be possible to demonstrate a morphological basis for all forms of endocrine activity; but, if ever this wide view of the animal economy becomes possible, it appears certain that the reticulo-endothelium will occupy a prominent place.

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Clinical Articles

Mummified Fœtus in the Cow—Cæsarean Section.

By J. G. WRIGHT and C. FORMSTON.

Royal Veterinary College.

THE subject of this article was an aged, crossbred Guernsey cow. Multipara. She was last served in January, 1930. We had not the opportunity of examining the animal until July 1st, 1931, when one of us (J. G. W.) was asked by the attendant to examine her, as she appeared to be calving. Hanging from the vagina was a portion of the placenta. It was chocolate-brown in colour, thickened and dry, but was not putrid. Examination *per vaginam* revealed the uterine

FIG. 1.—The Mummified Fœtus.

cervix to be three parts dilated, and beyond this the limbs of a fœtus could be palpated. These were dry and rigid, there being no liquor amnii. This was replaced by a small quantity of a dark brown thick glutinous substance. The thickened fœtal membranes entirely surrounded the fœtus, and upon it the uterus could be felt firmly contracted. The presentation can be seen from the accompanying photograph of the fœtus. A remarkable feature is that the head, which was flexed at the neck and lying in the left flank, developed under the umbilical cord, the latter structure passing from the calf's abdomen over the fœtal neck immediately behind the occiput. It exerted sufficient pressure to leave a definite impression in the mane and skin of the neck. This can be seen in the photograph.

One speculates as to whether this rigid fixing of the calf's head was in any way responsible for the abnormally long period of gestation

(eighteen months). Traction upon the fœtus resulted in a drawing back of the whole of the genital organs, and although a considerable time was spent in an effort to deliver the cow, no progress was made. As she resented forcible interference, it was decided to induce anæsthesia of the posterior limbs and genitalia by epidural injection, the technique of G. B. Brook being followed. Twenty c.c. of Waite's "procaine" was injected into the epidural space between the first and second caudal bones. Within a few minutes the tail became limp, and the posterior genitalia anæsthetised. Skin anæsthesia did not extend in front of the external angle of the ileum, and the cow, although staggering, remained standing.

An extensive examination was now made without resistance, and this only served to show the futility of further traction or of embryotomy, and thus it was decided that the only chance of saving the cow's life lay in the early performance of abdominal section. The cow was cast on the left side. The two under legs and the upper fore leg were hobbled together, whilst the upper hind leg was drawn well back to fully expose the site of operation. This was clipped and shaved, and finally prepared with ether and tinct. iodi. mitis, sterile towels being placed around the site held in position by towel clips.

General anæsthesia was induced with chloroform (B.D.H.) by means of a Cox's inhaler Two ounces were used as the initial dose and continued by the further administration of half an ounce about every five minutes. In all, six ounces of chloroform were used, the operation taking fifty minutes to perform. Anæsthesia was good. An incision twelve inches long was made in the flank, commencing six inches below the external angle of the ileum and extending downwards and forwards, through the skin only (photograph 2). The respective layers of muscle were separated rapidly by blunt dissection with the fingers in the direction of their fibres down to the peritoneum, which in turn was incised. Up to this point practically no hæmorrhage had been encountered.

One was next confronted with the task of withdrawing the gravid uterine horn out of the abdominal cavity, and this constituted the most formidable part of the operation. Its weight, which is estimated at 50 lb., had caused it to fall right away from the abdominal incision, and it was necessary for two arms to be introduced into the abdomen up to the shoulders, to draw it up into the abdominal wound. The posterior part of the gravid horn, near its junction with the uterine body, was drawn out of the abdominal opening and held there. An incision ten inches long was made through the uterine wall (on that

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side opposite the attachment of the broad ligament) exposing the fœtal head, and through this the fœtus and placenta were extracted.

The uterine wound was closed as rapidly as possible, first by means of a continuous suture (No. 6 silk) through all coats, and finally by a layer of interrupted sutures, including the serous and muscular coats. The viscus was then returned to the abdomen. The incision in the abdominal wall was next closed with four layers of interrupted sutures, closing the peritoneal and muscle wounds, and being of No. 6 silk, and those in the skin of quarter-inch tape. Tympany of the rumen rendered closure of the abdominal wound somewhat difficult; paracentesis, however, was not performed. The cow rose to her feet fifteen minutes after the completion of the operation, and one hour later was observed eating hay.

FIG. 2.—Showing the site of incision.

Second day: Animal comfortable, moving freely, eating, drinking, and chewing cud; temp. a.m. 102° F., p.m. 102.4. Third day: Eating and chewing cud; temp. a.m. 101.8, p.m. 102.4. Fourth day: Only picking food; moving freely, but grunting; temp. a.m. 101.8, p.m. 102.4; pulse 60. Fifth day: Still picking food, but respiration blowing; temp. a.m. 102.8; p.m. 102.8. Sixth day: No appetite; respirations further distressed, but pressure on the abdomen does not cause pain. The case is now suspected to be one of post-operative pneumonia; there is no cyanosis; temp. 102.8, p.m. 103; mustard is applied to the chest wall. Seventh day: condition the same; temp. a.m. 103.4, p.m. 103.6; one quart of normal saline and oxygen injected subcutaneously. Atropinæ sulph.; strychninæ sulph, *ā ā*, gr. ½; strophanthin, gr. 1/10; injected subcutaneously. Eighth day: Condition unchanged; temp. a.m. 103.6, p.m. 103.4; hypodermic

injection repeated; oxygen injected into the left pleural cavity. Ninth day: Animal decidedly worse; marked respiratory distress; grunting; temp. 104; oxygen injected into the right pleural cavity. Shortly afterwards the animal staggered a few times and then fell to the ground to die immediately.

The operation wound during this period was healing by first intention, and no dressings were applied other than twice painting it with tinct. iodi. mitis. on the second and fourth days. The animal had remained standing from the third day after operation. The fatal termination of this case was disappointing, particularly in view of the fact that the post-mortem examination revealed that, from a surgical standpoint, the operation was highly successful, there being no evidence of sepsis whatever.

Appended is the report of Dr. Hare, who carried out the post-mortem examination.

The carcase was in good general condition. The laparotomy wound in the right flank had united and was healthy, the omentum being adherent to its deep aspect. The operation wound in the body of the uterus was also healthy and united, and the omentum was adherent to its peritoneal surface. The uterus had involuted normally, and its lumenal surface was highly injected, but showed no ulceration or suppuration. Its lumen contained a small quantity of thick fluid containing altered blood. There was free passage through the cervix, which was swollen and congested. The vagina was slightly congested. The bladder, urethra and ureters appeared normal. The cortex of both kidneys was slightly paler and softer than normal, and showed faint radial striations, apparently tubules, in a state of albuminous degeneration. The medulla was highly congested; the capsule stripped readily. The liver was slightly swollen, and the cut surface showed prominent greyish-opaque central zones of the lobules. There were irregularly scattered ecchymoses in the mesentery, which was unusually fat; a golf-ball-sized lipoma was attached by a pedicle about half an inch long to the ileum, about four feet from the ilo-cæcal valve. Owing to the subcutaneous injection of oxygen, the tissues of the mediastinum were exceedingly emphysematous, and the emphysema had extended along the retroperitoneal tissues of the root of the abdomen as far as the brim of the pelvis. It had also extended into all lobes of the lungs as a most pronounced interstitial emphysema. No other abnormality was found in the respiratory tract. The heart was of normal shape and size, all cusps of the tri-cuspid valve contained ecchymoses with slight swelling, and of recent

formation. There were thickly scattered ecchymoses in the outer coat of the root of the pulmonary artery extending as far as its bifurcation. Appearances (macroscopic) suggest delayed chloroform poisoning.

The Gall-Bladder.

By W. P. BLOUNT, F.R.C.V.S.
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TEXT-BOOKS on physiology do not give any account of the functions of this organ, and it is quite unusual for lecturers to include this subject in their discourses, probably because any knowledge concerning it is scarce and widely scattered in scientific literature.

In order to consider whether the gall-bladder empties mechanically or actively, and whether it has any function other than that of storing bile, it is necessary to review experiments and data with an open and generous mind.

Anatomy.—The mucosa of the gall-bladder is of a honeycomb appearance, occasioned by periods of relaxation and distension, and it is suggested that bacteria are harboured in these mucous crypts awaiting a suitable moment for producing infection. There are three layers of muscles, and these lie strongest in the neck of the bladder. The wall is richly supplied with muscles, and has little elastic material, which favours the view that the organ empties by active muscular contraction and not by passive elasticity. The blood supply is carried to the liver, and there is an even closer relationship with the liver by means of certain lymphatic elements, probably evidence of the presence of reticulo-endothelial cells. The vagus and sympathetic nerves innervate it in man, but it is uncertain if this applies to all animals.

The cystic duct is rich in glands, elastic and nerve fibres, and at the neck of the bladder the mucous membrane is thrown into folds known as the *valvuli heisteri*. These and the possible presence of an actual bladder-neck sphincter (Lutkens, 1926), have been associated with biliary stasis, because a small mucous plug may prevent the emptying of the gall-bladder; this is known as muco-stasis (Berg, Ergeb d. Med., Strehlenforschung).

The common duct of the biliary system ends in the duodenum (after passing through the head of the pancreas), by means of a *papilla vateri* surrounding which is a sphincter of circular muscle fibres,

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described by Oddi in 1887. In the guinea-pig there is a special pouch situated at the lower end of the common bile duct, but apparently this takes no essential part in the emptying of the gall-bladder. It can be appreciated therefore that should the pancreas become severely congested, or the duodenum become hypertonic in its muscular action, or the duodenal mucosa become inflamed (catarrhal), there will be an impediment to the flow of bile, which will probably be followed by jaundice.

Boyden (1926) found five cases of duplication of the gall-bladder in man out of 19,000 examined ; whereas in his examination of 10,000 domestic animals, duplication occurred 1 : 8 cats, 1 : 28 calves,1 : 85 lambs, and 1 : 200 pigs.

Physiology.—Some writers have assumed that because there is no gall-bladder in the horse, deer, rat, or dove, such organs are unnecessary, and further, that they may be removed surgically indiscriminately, because if we have no reservoir for bile in the horse, there is no need to have one in man. It is known, however (Held and Goldbloom, 1928), that in those animals which are without a gall-bladder, large dilated, intrahepatic ducts function in its place. Following cholecystectomy these intrahepatic, and even the common bile ducts, dilate very considerably, endeavouring to compensate for loss of the original organ (Judd and Counsellor, 1927). Cases are recorded where the gall-bladder has been contracted and entirely filled with stones, following which the common duct dilated sufficiently to function in place of the original bladder. This means that, unlike the heart and lungs, the gall-bladder is a dispensable organ. This is important, because if it be removed before compensation has occurred in other tissues, much pain may ensue. Thus Mayo has shown that to cut an undiseased organ (gastro-enterostomy) worse symptoms may ensue when there is no ulcer present, because no accommodative compensation has occurred. Similarly with the gall-bladder. When it becomes diseased the process is nearly always sufficiently slow to allow suitable compensation by intrahepatic ducts ; if, however, the organ is removed whilst still healthy, no such compensation has occurred, and pain and other sequelæ follow.

There is abundant evidence to show that the gall-bladder can act as a reservoir for bile, but there is also work which points to the fact that bile, whilst present in the bladder, undergoes dehydration with consequent concentration and darkening of colour. In addition to the absorption of water, lipoids, pigments, and certain salts are also picked up by the mucosa ; this function can also apparently be performed by the common bile duct, and also by the larger intra-

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hepatic ducts. Presumably this is what occurs in the horse. Whether concentrated gall-bladder bile has efficiency greater than bile obtained direct from the liver is uncertain, but some workers assume that concentrated bile is much more effective in aiding fat digestion and assisting peristalsis. During digestion bile from both sources will enter the intestines; in all probability bladder bile will predominate during the early part of duodenal activity. During the dehydration of bile in the gall-bladder a concentration from twenty to thirty, and even up to ninety, times that of the liver bile occurs, hence the dark colour. After removal of the gall-bladder the common duct assumes its function; this is not so with the cystic duct. One of the most important constituents affected by dehydration is cholesterol; after excess fat is ingested cholesterosis occurs; this may be so great as to interfere with the absorption of other substances like pigments, and with the consequent biliary stasis cholelithiasis may occur. Peel, studying types of gall-bladder stones, classified them as follows: (1) true cholesterol; (2) cholesterol-pigment lime; (3) pigment stones; (4) carbonate pigment stones; (5) calcium and magnesium stones; (6) atypical cholesterol stones. He considered that the copper content of bile was important in stone formation.

Factors Associated with Emptying of the Gall-Bladder.— It is generally agreed that there is periodic emptying of the gall-bladder, but the factors concerned in this are disputed, chief amongst which are: (*a*) muscle of the bladder; (*b*) elastic fibres of the bladder; (*c*) duodenal peristalsis; (*d*) the sphincter of Oddi; (*e*) movements of the diaphragm; (*f*) vegetative innervation; (*g*) dietetic; (*h*) chemical.

Meltzer was one of the first investigators of gall-bladder function; he discovered that 33 per cent. $MgSO_4$, applied directly to the sphincter of Oddi, might relax it and cause contraction of the bladder; this he associated with the innervation of the organs concerned. Many workers have shown that the presence of fat in the duodenum is one factor which brings about the passage of bile from the bladder to the duodenum, but Sosman and others (1925) demonstrated that although pure olive oil in the intestines reduced the size of the gall-bladder in dogs, this was not the case with man. Higgins and Mann (1926) gave a meal of cream and egg yolk to amphibia, fish, birds, and mammals, and in each case the bladder emptied itself within one and a-half and three hours, due to active contraction of the muscular coat. They decided after experimentation that respiratory movements, the sphincter of Oddi, and intra-abdominal pressure play only a subsidiary part in the mechanism of gall-bladder con-

traction, and demonstrated that the gall-bladder could remain full even though the sphincter of Oddi was relaxed. Trommer (1927) found that glucose or magnesium sulphate per duodenum caused contraction of the gall-bladder, which was followed by a flow of bile into the intestines. Fats and $MgSO_4$ were considered to relax the tone of the duodenal muscles, but a second method for obtaining a flow of bile was by using pituitrin, which increased duodenal peristalsis (Burget, 1926). Higgins and Mann said that in dogs muscular waves passed over the gall-bladder at ten to forty second intervals, but Whittaker (1926), examining cats and dogs, saw no peristalsis in this organ except in the ampulla ; they did not think that nerves assisted the excretion of bile from the bladder. Fats in the duodenum or small intestines were, however, effective. In the dog, section of the sphincter of Oddi did not prevent the gall-bladder filling, but an opposite effect was seen in the cat. Kodama (1926) believed that the gall-bladder contracts by its own elasticity after there is first obtained a relaxation of the sphincter, although the elasticity is insufficient to completely empty the bladder. Higgins (1927), however, contended that the intrinsic muscles are of more importance. Burget (1927) finally decided that the bladder plays only a passive rôle in controlling bile flow by reason of its elasticity, and the intra-abdominal pressure. The main factors were believed to be the tonicity and peristalsis of the duodenum. Boyden and Birch (1927) found that after severing the bile duct from the duodenum in a cat, the gall-bladder still continued to empty itself ; no evidence was found to support the theory that the mechanical passage of food emptied the bladder.

To assist in the elucidation of this problem, cholecystography has been employed, and valuable information gained. By this means (aided by preliminary meal rich in fat) stones of the gall-bladder have been seen to move as bile was expelled ; the reduction in size of the bladder took from half to one hour.

In favour of the passive relaxation of the gall-bladder whilst emptying itself, Haberland (1924) suggests that the pressure of the surrounding organs plays an important part. Ivy and Oldberg (1928) imply that a duodenal hormone (which they term cholecystokinen) is necessary for bladder relaxation ; they assume that it is very similar to secretin, being liberated by the duodenal mucosa after acid from the stomach reaches it. They satisfied themselves, by cross circulation experiments, that such was the case. Opposed to the passive nature of this act, we find Higgins and Mann stating that the gall-bladder of the pike empties satisfactorily in spite of the fact that it has no

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diaphragm, and breathes by gills. Careful dissection has shown that the gall-bladder is supplied by the vagus nerve, whose function is that of producing both motor and secretory activity to it; this nerve also innervates the bile ducts. Westphal (1927) has shown that the sphincter of Oddi acts like the pylorus in that it prevents the passage of fluid contents until it is relaxed. Nicotine increases the tonicity of the sphincter, whilst adrenalin causes dilation. Pathologically it is known that should calculi occur in the common bile duct, or in the cystic duct, a compensatory muscular hypertrophy of the gall-bladder takes place; this may be followed by jaundice, a feature also seen if spasm of the sphincter of Oddi occurs.

It can be seen from the facts presented that some recent writers are unable to confirm the findings of previous experimenters, which leaves the question of gall-bladder contraction still unsettled, but generally speaking it would appear fair to presume that each of the eight factors previously mentioned may play a part in emptying the gall-bladder. Certainly some of these factors are definitely of secondary importance, namely the respiratory movements, and elastic fibres of the bladder; but whether there is an inherent contraction of the organ similar to that of the heart (controlled by the vagus and sympathetics), or whether an autacoid substance is necessary to initiate gall-bladder contractions, is still uncertain.

Clinically it is important to open the gall-bladder at each post-mortem on a domestic animal, more especially if there are no major lesions in any part of the body which could account for death. Notes might be taken with advantage concerning the consistency and colour of the bile, the character of the gall-bladder, with particular reference to its mucous membrane and general muscular tone; and if "stones" are found, their characteristics should be recorded.

At one time it was said that fats should be avoided when canine patients were suffering from icteric complaints, but if the type of jaundice be other than obstructive, a fat meal would appear to be advantageous, since it is generally admitted that the presence of fat in the duodenum leads to emptying of the gall-bladder. Fat, however, tends to depress gastric function, so that fat meats are not indicated, otherwise the albuminous portion will be undigested; rather try milk with high cream content, non-fatty constituents of milk needing but little digestion.

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Vomiting in Ruminants.

By J. T. EDWARDS, M.R.C.V.S.

Addlestone.

THE writers of the early text-books on veterinary physiology as a rule declared that vomition did not occur in ruminants, but the observations of clinicians has proved that it can and does occur, though in much less frequency than in other animals. The occurrence of the act once established, various veterinarians tried to account for the working of it by supposing that it was brought about by a compression of the stomach between a contracted diaphragm and adbominal muscles, with a resultant forcing of some of the contents to the outside, *via* the œsophagus.

Begg,[3] in Wallis Hoare's "System of Veterinary Medicine," states that the vomiting centre in the medulla is not easily roused in ruminants but can be stimulated by afferent messages from pathological conditions of the throat and œsophagus. Vomition sometimes occurs after ingestion of fermenting food and of rhododendron, and in connection with the latter, Wester states that vomition alternates with regurgitation. Begg has produced vomition in recumbent cattle affected with tympany by drenching them with magnesium sulphate.

The method of direct examination of the cardiac region of the ruminant stomach by means of the fistula in the rumen, now employed extensively by all workers in the field of ruminant digestion, has been utilised by Wester in a study of vomition. He found that young

cattle can regularly be made to vomit if they are dosed with about 2½–3 drachms of pulv. veratri alb., given in a vehicle of half a pint of a 10 per cent. aqueous solution of sodium bicarbonate.

Now solutions of sodium salts, of sugar, and also blood and milk, exert a strong action on the œsophageal groove; they cause closure of it when given *per os* (see Wester[2]), and drugs, etc., given in such go to the omasum and abomasum. Veratrum given in this way sets up after an interval, probably spent in traversing the omasum, a reflex action which results in vomition, which action, according to Wester,[1] is very similar to that of regurgitation. He thinks that the œsophagus is practically exclusively responsible for the act, which takes place at a period of inspiration with the glottis closed, and accompanied by strong contraction of the abdominal muscles. The latter do not, however, contribute to the ejection of food, as the rumen and reticulum are quite static during the act, nor is food matter squeezed out in any way. The hand in the cardiac region feels a strong sucking action at the œsophageal opening. Whether the abomasum contracts is evidently not known. Wester does not allude to this point, and evidently does not consider it significant, and in any case the omasum would probably offer an efficient barrier to a backward flow of ingesta through it. What happens is that the œsophagus contracts in its longitudinal fibres, and the cardiac extremity becomes dilated, assuming a funnel-shaped form, and that, together with negative thoracic pressure, results in a like pressure in the distal extremity of this organ, and a large amount of ruminoreticular contents is sucked up, and by anti-peristalsis is carried up and ejected through the open mouth.

Wester considers that the acts of vomition and regurgitation are essentially the same. The main points of comparison are these:

Vomition.	*Regurgitation.*
Involuntary and accompanied by nausea.	Voluntary.
Fore stomachs inactive	Fore stomachs active.
Head well stretched out.	Head not markedly extended.
Abdominal muscles strongly contracted.	Abdominal muscles only momentarily contracted.
Large amount of food sucked out.	Smaller amount of food sucked out.

Vomition occurs less readily in sheep and goats than in cattle. With regard to practical application, vomition would seem to be desirable in cases of choking—impaction of the œsophagus, but whether veratrine has the same effect when given intravenously or

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subcutaneously, as when given through a closed œsophageal groove, I do not know. At any rate, it is quite easy anatomically to inject drugs by a Record syringe directly into the abomasum. An overloaded rumen might with advantage be lightened by inducing vomition. It is here relevant to mention that reflex closure of the œsophageal groove is only obtainable with certainty in cattle less than about two years old, and that the presence of digestive disturbance interferes with the closure, and that the use of the stomach tube also prevents it. Evidently the act of swallowing has a part in the mechanism of reflex closure.

Incidentally, if anyone is inclined to attempt direct intra-abomasal drug administration (this has already been used in South Africa in connection with worm infestation in sheep), the excellent monograph of Lagerlöf, " Investigations of the Topography of the Abdominal Organs in Cattle, etc.," published in English in the *Skandinavisk Veterinar-Tidskrift*, Vol. 19, pp. 253–365, with eighty figures and photographs, is warmly recommended, and should be of immense value to all engaged in cattle practice. It is probable that the solutions which excite the œsophageal reflex have a more energetic action when given hot or warm than they have when cold. Tartar emetic does not cause vomition in sheep and goats, and the same applies to cattle; instead, it causes gastric disturbance and diarrhœa.

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Reviews

Selected Readings in the History of Physiology. By John Farquhar Fulton, M.D., Stirling Professor of Physiology, Yale University School of Medicine. London: Baillière, Tindall and Cox. 1930. 61 Illustrations. Pp. xx+309. 22*s*. 6*d*.

IN this work we find a companion volume to that published on " Readings in the History of Pathology," 1929, edited by E. R. Long. Although it covers in period of time authors from Aristotle, 384 B.C., to Magnus, 1927, it is not a continuous history of physiology, but the chapters are arranged by subjects including The Circulation of the Blood, Capillaries, Respiration, Digestion, Muscle, and Peripheral Nerves, and the C.N.S.; two other chapters, the first and the last, are devoted to General Principles

of Physiology, and " Miscellaneous " respectively. In each of the eight chapters the various authors are arranged in chronological order; terse historical or biographical paragraphs precede the actual description of the individuals' classical work.

It is impossible in a short review to describe a book adequately, but veterinarians can rest assured that there are in it many passages of intense interest, for example, in the account of Steven Hales' experiments (1677–1761) we find reference to " determining the blood pressure by inserting a tube into the femoral artery of a mare, which he had ' caused to be tied down ' " ; and again, in Hales' eleventh experiment there are numerous references to vivisection on dogs. Boyle and Hooke's experiments on respiration also receive attention, but as the majority of physiological facts have been determined by animal experimentation, it is natural that such an attractive history of the science contains passages directly relative to our profession.

In contrast to " Readings in Pathology," we have accounts of several physiologists whose contributions to science were made during the present century, namely, those of Starling, Haldane, Barcroft, Lewis, Gowland Hopkins, Sherrington, Bayliss, Adrain, etc. Some readers would be quite content with the views and experience of writers of the past, but on the other hand, by including modern writers, the editor has brought right into one's grasp an active link between the old and the new, which is both interesting and valuable. Pavlov's own description of his operation whereby gastric juice uncontaminated with food was obtained from dogs is useful as well as historical; after many attempts it took him one and a-half to two hours to complete the operation.

Finally, one word must be said concerning the memorable work of Oliver and Schafer on experimental endocrinology. Extracts were made from the suprarenals of sheep, calves and dogs; injections were made into dogs, cats, rabbits and frogs, and the results and principles involved have been used successfully in man. Could we have a finer example of the importance of veterinary physiology? Like its companion volume, it is attractively bound in red, printed on excellent paper, and as the accompanying illustrations are very well reproduced, it becomes a worthy addition to any library.

Recent Advances in Hæmatology. By A. Piney, M.D., M.R.C.P. Third edition. London: J. and A. Churchill. 1931. Pp. viii+348, with 4 Coloured Plates and 18 Text Figures. 12*s.* 6*d.*

In view of the fact that the study of blood is not as popular as many other branches of biology, it must be very gratifying to author and publisher alike to realise that another edition has been necessary in so short a time. It retains in general the characteristics of the Recent Advances Series, but several small important alterations have been made to the second edition.

Figure 1 has been withdrawn to be replaced by a much better specimen illustrating the spleen in Gaucher's disease. Chapter 2, on the Development of the Blood Cells, makes delightful reading, and gives a clarity of thought and expression to a very complex subject. To the chapter on Pernicious Anæmia has been added several more paragraphs dealing with treatment, which practical aspect will appeal to the majority of readers,

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especially in view of the fact that " treatment of pernicious anæmia is now very satisfactory, and great improvement can be recorded." The two following chapters are entirely new, and deal with " Some Anæmias with Low Colour Index," and " Sickle Cell Anæmia " respectively. The Arneth and Polynuclear Counts receive due prominence, and new matter has been added concerning the effect of Blood Irradiation. To the diseases of the reticulo-endothelial system has been added a note on Mikuliez's disease. The four coloured plates are, as usual, really valuable; as a fact, there are really five, because Plates III and IV of the second edition have been suitably combined. The new plate depicts various types of early premyelocytes ; it is an excellent picture, but unfortunately some of its value is lost because the cells are unnumbered, which detracts from the usefulness of the accompanying descriptive text. No doubt this slight error will be corrected, for the plate is well worthy of its position in the book.

In the last edition the Index was unfortunately hidden, but this has now been remedied. Appendix I, on " Some Notes on Hæmatological Technique " is amplified. Additional paragraphs on Epstein's Oxydase Reaction, Leon's method for staining Amato's bodies, Measurement of R.B.C.'s by halometric methods, complete it. The only complaint, if one may call it so, concerns the late Appendix II, which described briefly the numerous hæmatological text-books in the author's library. To some at least it was almost as valuable as the glossary or index, but apparently the publishers have decided to omit it for lack of suitable space. No mention is made of the Guttadiaphot, but as these papers have not yet received credit, other than in Germany, the author is probably correct in delaying their criticism and description.

The Meat Industry. By D. J. ANTHONY, M.R.C.V.S., D.V.S.M., Chief Veterinary Officer, Brierley Hill U.D.C. ; Messrs. Marsh and Baxter, Ltd. ; Lecturer, the Brierley Hill Institute ; and W. G. T. BLOIS, M.B.E., F.I.S.A., General Secretary the National Federation of Meat Traders' Associations (Inc.) ; Lecturer on Business Organisation and Trade Law to Meat Trade Classes. Second edition. London : Baillière, Tindall and Cox, 1931. Pp. xii+256. *7s. 6d.*

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IT is with pleasure that we see that a second edition of Mr. Anthony's " The Meat Industry " has been published, and the collaboration of authorship of Mr. W. G. T. Blois, who is well known throughout the meat trade, and is secretary of the Meat Trades' Federation, gives it an added value. There was a very real need for this book, which was the first in its field. Although primarily designed for the use of student butcher classes, for whom it is intentionally and primarily written, it contains so much information on the subject, that it cannot but be of use to veterinary graduates who are either studying for or are engaged in the meat industry. Our contemporary, the *Veterinary Record*, expressed the opinion, when the first edition was published, that " any veterinary inspector who is not already familiar with the contents of the book is not familiar with his work." We thoroughly endorse this opinion, and we commend " The Meat Industry " as a book which should find a place on the bookshelf of every veterinary surgeon whose duties include meat inspection, and, indeed, anything to do with the meat trade.

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